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Dr. Bushland
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Entomology Research Division
Kerrville, Texas

Liquid medium for screw-worm rearing

**W. C. McDuffie, Chief
Insects Affecting Man and
Animals Research Branch
Beltsville, Maryland**

November 8, 1963

At the time of my visit to Mission on November 1, I saw screw-worm larvae being grown on the liquid medium which the Methods Development Section has devised. I was strongly impressed in two ways, (1) that the basic idea is very sound, and (2) that the people presently working on it badly need some expert help.

Mr. A. J. Graham and Mr. Frank Dudley deserve a lot of credit for the work that they have done so far. If the liquid medium (hydroponic culture is the term they have been using) can be perfected, it appears that it will be possible to more than double the capacity of the rearing vats. This, in turn, means that the capacity of the plant could be doubled by merely modifying the plant, and that the cost of producing sterile flies could be greatly reduced. At the same time, the many headaches connected with the procurement of fresh meat and blood could be eliminated. Since it now appears that far more sterile flies will be required for Southwestern screw-worm eradication than was originally contemplated, a drastic improvement in production could save the program.

Our research group has not participated in the development of the liquid medium, mainly because the idea originated with Methods Development and they preferred to develop the idea without help. However, Mr. Baumhover and I discussed the present status of the medium yesterday with Drs. Wilbur and Cartman. We offered Dr. Gingrich's services as a consultant, because I feel that he probably already has sufficient information on screw-worm nutrition to be able to supply the finishing touches that are needed to perfect the liquid medium. The ADE people indicated that they would very much like to have Dr. Gingrich's help, so he will go down to Mission next Tuesday to look over the work that has been done so far and help in planning further tests.

O. H. Graham

**O. H. Graham
Investigations Leader
Livestock Insects Investigations**

cc:

R. C. Bushland ✓
A. H. Baumhover

To duplicate

NOV 12 1963

Entomology Research Division
Kerrville, Texas

Liquid medium for screw-worm rearing

November 8, 1963

W. C. McNeill, Chief
Insect Attraction Unit and
Animal Research Branch
Belleville, Maryland

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Mr. A. J. Graham and Mr. Frank Bailey deserve a lot of credit for the work that they have done so far. If the liquid medium (hypocotyl medium) in the form they have been using) can be perfected, it appears that it will be possible to grow more than double the capacity of the rearing vats. This, in turn, means that the capacity of the plant could be doubled by merely modifying the plant, and that the cost of producing sterile flies could be greatly reduced. At the same time, the many headaches connected with the procurement of fresh meat and blood could be eliminated. Since it now appears that for more sterile flies will be required for Southwestern screw-worm eradication than was originally contemplated, a drastic improvement in production could save the program.

Our research group has not participated in the development of the liquid medium, mainly because the idea originated with Methods Development and they preferred to develop the idea without help. However, Mr. Bannister and I discussed the present status of the medium yesterday with Dr. Miller and Graham. We offered Dr. Gingham's services as a consultant, because I feel that he probably has sufficient information on screw-worm nutrition to be able to supply the finishing touches that are needed to perfect the liquid medium. The ABE people indicated that they would very much like to have Dr. Gingham's help, so he will go down to Mission next Tuesday to look over the work that has been done so far and help in planning further tests.

W. C. McNeill

W. C. McNeill
Investigations Leader
Livestock Insect Investigations

cc:
W. C. McNeill
A. E. Bannister

Dr. Gingham

HYDROPONIC DISCUSSION

January 14, 1965

10:00 A.M.

Following conclusion of the regular staff meeting, Dr. Gartman stated that in line with our discussion and suggestion made last week, we want to begin by making a list first of the problems or differences which we don't understand about hydroponics. Dr. Meadows pointed out that some of the things we are most concerned about may develop not to be actual problems.

The first problem was determined to be the SMALL SIZED WORMS. While last summer, in August, the "hydro" flies averaged 64 mg. in size, the overall size of our production has gradually gone down as we have switched over to the liquid rearing process this fall, with an increase in the percentage of extremely small worms believed due mainly to being pushed off the vats. The amount of real small worms destroyed has decreased as various remedies have been instituted so that loss in production has been of no serious consequence. A further evidence of this is that while in the last 4-5 weeks lung production has been cut several times, there has not been a corresponding drop in the production level indicating a higher yield on hydroponics possibly because of better care through employees' adaptation to the new rearing process. Whether or not size in itself is a serious problem has not been determined as SAG tests show them comparable to the lung-reared flies and the 14-day mortality tests continue to hold up. However, on the more recent Cage Ranch trapping studies we did not catch as many of the small "hydro" as meat-reared flies, and in Poza Rica we got 9 sterile to 1 fertile female in the traps. We don't really know the significance of this ratio. Dr. Hightower said they are presently running some tests on competitive mating but have only run it one time so far. However, we have also been taking some dry weights and the dry weight of the animal-reared males is roughly three times the weight of the colony flies. They are dried to eliminate the changes in the water content of the fly as this is the most uniform method of weighing. This, however, may or may not mean anything.

The second problem was decided to be the CHANGED RATES IN DEVELOPMENT. Mr. Sudlow stated the hydroponic larvae are slower than the meat-reared in larval development taking about a half day longer to complete the feeding cycle, but are faster in the pupal development. In this connection, he further stated that if insects are nutritionally starved in the larval stage, it will affect pupal development, often causing pre-emergence. Dr. Meadows stated the rate of pupal development has increased about 24 hours with irradiation now being accomplished at the 5-day age while previously this was done at 6 days, though biologically they are the same age. These flies are also emerging earlier which has affected release date so that instead of releasing flies the ninth day after pupation, we are releasing the eighth day and in some instances on the seventh day.

25 gal. honey - need 55 gal. honey.

The third problem was considered to be the EXTENDED RATE OF EMERGENCE WITHIN THE SAME GROUP. Mr. Blackwelder stated there is at least a 24-hour variation in pupal age in every canister irradiated, and Mr. Sudlow said they also get the same widening emergence in the colony. Egging time has to be fairly constant in the Fly Colony and this may be the reason for our erratic egg rate.

The fourth problem was stated as REDUCED EGG PRODUCTION. Our selection pressure which was instituted around June of last year and originally gave us a female-male ratio of 60-40 or better in the Fly Colony with a 39% increase in eggs and also a one-third reduction in number of cages required, is no longer providing a greater number of females. This selection originally was placed on the larvae coming off the vats during the first eight hours over the water collection. However, when it became evident we couldn't collect enough during this period for colony restocking and to get bigger flies, selection was extended to include the second eight hours. Several weeks ago when a noticeable reduction in egg production became evident, a check was made to determine the female-male ratio and it was found to be about 50-50 and since that time it has remained constant at about this ratio.

Problem number five was determined to be the ERRATIC CRAWL-OFF. By this we mean the fact that crawl-off is being accomplished by unnatural means rather than when larvae are full-grown and have completed feeding. Some unnatural means noticed are their being pushed off either because cotton too near vat edge, "boiling" which could result from various conditions - mechanical, environmental, etc., overcrowding, or when there is a time lag in adding food following vacuuming of spent media.

In reviewing the five problems enumerated above, it was the consensus of the staff that the first three are the ones of main concern and the remaining two are considered tied in with these. It was also generally agreed that if we can find a solution to the first problem, it will solve the remaining four problems.

There are three major factors we need to consider in finding solutions to these problems which are indicated on the attached outline as NUTRITION, GENETIC AND ENVIRONMENT. Since there have been some changes both in the diet and in the method of feeding, these nutritional aspects have been broken down minutely for further study. The diet that produced the largest worms was composed of dried blood, fish flour (mink grade) and cottage cheese. Since Dr. Gingrich at Kerrville did some work in defining what should be in the diet, we might want to have him come down and study our diet composition including the substitute products we are now using to determine if it is nutritionally adequate. Some felt that while evaluation of other dried products tested to find the best cheap media did not show any significant difference, the addition of a number of substitute products with only slight variations have combined over a period of time to give us a smaller sized worm.

At this point there was considerable discussion of the method of our sampling in determining larval and pupal size, the question having been raised at the Hyattsville level by Dr. Beal of ARS Biometrical Services. Dr. Meadows felt that since Dr. Beal is both a Statistician and a Geneticist, he could give us some valuable assistance in solving our hydroponic size problem and we ought to invite him down. Dr. Williams stated Dr. Beal had expressed an interest in statistically analyzing this for us and he felt he could probably come immediately if we wanted him.

We have recently incorporated two new changes in our method of feeding as result of tests conducted by Methods Development. They are that it was better to feed the hydroponic larvae every two hours than at less frequent intervals; also, that it was better to put warm media on the vats.

In addition to the liquid feed, a dry mix composed of fish and milk protein has been sprinkled on the surface at scheduled intervals and as needed to calm worms when "boiling" and excessive crawling noted.

To determine if there has been a genetic change in our production we must consider the selection pressure which we instituted last June primarily for the purpose of getting a larger percentage of females for the Fly Colony. Dr. Hightower pointed out that we are selecting for two things: those coming off first (accelerated development) and for those able to complete development on liquid media (i.e. do not "boil off.") We are now collecting our Fly Colony pupae from hydroponic production only. Still we are putting worms from the colony back on lung even though the environment for the meat worms is entirely different from the "hydro" worms. Dr. Hightower felt a quick method by which we could determine if there has been a genetic change in the plant stock would be to take the five Texas ENT strains of screwworm flies which range from the second to the fifty-third generation in colony and put them with plant stock on the same diet and analyze the larval and pupal development. Dr. Gartman said that when we study this further, we may decide this should be our next approach.

Asked if there had been any other changes in the colony, Mr. Smith stated flies were egged on the eighth day but now it is done on the seventh day though it varies back and forth which was considered normal. The egging attractant, however, has been changed from whole blood or incubated blood albumin to spent hydroponic juice.

The environmental items that need further study were considered to be mainly self-explanatory. We know that "boiling" can be a symptom of "hot spots" in a vat either from vat heating coils or larval body heat. We have experimented with extended vat sides (depth of vat) to prevent cotton coming to the very edge and allowing larvae to be pushed off. But, while it kept larvae longer on the vat, it did not materially affect larval size. The installation of fans was primarily for the

purpose of causing worms to "dig in" and stay on the vats longer. As has been stated in many previous discussions, all of these various things we have tried have just been treating the symptoms until we can determine the cause of our problems.

An important factor in the hydroponic media is its consistency. If too dry or sticky, larvae don't feed properly and when this condition is noticed, the media is sprayed with warm water.

Methods Development have run several tests with from five to 15 grams of eggs per vat to determine the number of larvae to rear on a vat. Twelve grams has been the standard number used on hydroponics as tests with smaller amounts did not affect size.

Intensity of care is all-important as Mr. Smith guarantees small worms will be the result of an inadequate staff to properly care for the larvae. Of course, the longer our employees work with this new rearing process the more proficient they become and this is evidenced by the fact that production has remained high despite many recent cuts in the lung production which now only involves 12 vats per shift.

Discussion ended at 12:00 noon.

HYDROPONIC PROBLEMS

- I. SMALL SIZED WORMS
- II. CHANGED RATES IN DEVELOPMENT
- III. EXTENDED RATE OF EMERGENCE WITHIN SAME GROUP
- IV. REDUCED EGG PRODUCTION
- V. ERRATIC CRAWL-OFF

FACTORS TO CONSIDERA. Nutrition

1. Diet

- a. Blood Protein
 - (1) Fresh
 - (2) Dried
 - (3) Plasma
 - b. Fish Protein
 - (1) Fish Flour
 - (a) Mink Grade
 - (b) Commercial Grade
 - (2) Fish Meal
 - (a) Menhaden
 - (b) South African Pilchard
 - c. Milk Protein
 - (1) Cottage Cheese
 - (2) Milk Supplement (Suckle)
 - (3) Nonfat Dry Milk (CCC)
 - d. Formaldehyde
2. Methods (Feeding)
- a. Frequency
 - (1) Liquid
 - (a) 2 Hours
 - (b) 3 Hours
 - (2) Dry
 - (a) 2 Hours
 - (b) 3 Hours
 - (c) As Needed

B. Genetic

1. Selection Pressure

a. Medium

- (1) Meat
 - (a) Horse
 - (b) Nutria
 - (c) Whale
 - (d) Lung
 - (e) Slunk
 - (f) Seal
- (2) Liquid

b. Accelerated Development

2. Genetic Drift in Colony

C. Environment

1. Temperature
 - a. Vat
 - b. Ambient
 - c. Water
 - d. Medium
2. Cotton on Vats
 - a. Staple
 - b. Thickness
3. Air Movement
 - a. Fans On
 - b. Fans Off
4. Consistency of Feed
5. Number of Larvae Per Vat
6. Intensity of Care

HB
350.

LB
 960

ST
980.

AA
 210

1. Consistency of food
 2. Number of leaves per lot
 3. Consistency of food
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Dr. Hightower

*File - Signed media
bearing other
reports*

February 16, 1965

To : Donald L. Williams, Chief Staff Veterinarian
Screwworm Eradication, ARS-AME

From : Victor C. Beal, Jr., Biometrist, Livestock Research Staff,
Biometrical Services, Federal Center Building
Hyattsville, Maryland

Subject: Report on Inspection Trip to Screwworm Eradication Plant
in Mission, Texas

While in Mission, I inspected the plant, quality control procedures, and the daily production records.

Ten-day averages were obtained from the daily pupae size records over the past two years. These ten-day averages were plotted on graph paper. This formed a curve that was fairly erratic with the largest pupae occurring when either horse meat or nutria was fed and the smallest pupae occurring when lung meat was fed. However, there were periods during which lung produced quite large pupae. This can be seen by looking at the top of the graph. This shows the proportions of the various ingredients fed. Prior to the present trouble, the smallest pupae appeared to come during the months of December and January. This may have been due to climatic conditions. The selection pressure for smaller flies was started about the first of June 1964. The main ingredient fed during this time was lung. The decrease in size since June has been fairly constant with the decrease being hidden in August when nutria comprised the main ingredient of the diet. After studying this graph, I formed the opinion that the decrease in size was in large part related to the selection pressure with very little relationship to the feeding of liquid media.

Mr. Graham and I had a conference at which Mr. Baumhover, Dr. Hightower, Dr. Davis, and Dr. Burnett were present. It was Mr. Baumhover's opinion that the decline in size might be a desirable thing. However, there was some disagreement about this hypothesis. It was my opinion, with the selection for smaller pupae and faster developing larvae, that the metabolic rate was speeded up perhaps with earlier sexual maturity and with flies that burned up food reserves more rapidly. I suggested several tests; one being a competitive mating test between large and small flies from the colony; a second test for differences between the plant strain and ERD strain following starvation; and a third test involving a cross-breeding study between the plant strain and the ERD strain on several diets. I definitely feel that a competitive mating test between large and small

plant flies should be carried out. Also, I feel that a suggestion made by Mr. Baumhover about more SAC tests between HAD and plant flies should be carried out.

On methods development, I suggested to Mr. Graham to make sure that his plant controls are next to his test vats for any comparison being made.

Dr. Hightower suggested that dry weights be obtained on some samples of larvae to see how dry weight compares with wet weight. I second this idea.

I made several suggestions to Mr. Graham relating to quality control. Dr. Davis had several suggestions on this subject which were helpful. I believe that he can be of some help to the Mission people in the future on this subject. I suggested that they continue to obtain the weights on samples of 100 larvae from each shift. This is a procedure which they started about a month ago. It was suggested that they continue to obtain duplicate samples and to weigh with more accuracy. Dr. Davis made the suggestion that they obtain weights more often for awhile. I concurred with this suggestion and recommended the collection of two samples hourly for two weeks. This is done at the water separator. This will indicate whether one set of duplicate samples from each shift is adequate.

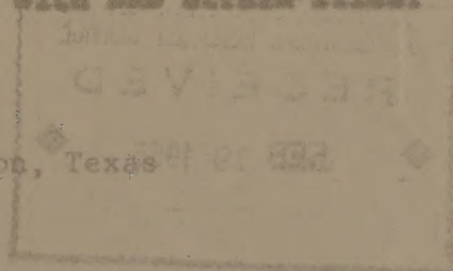
As you know, they obtain counts on pupae size by counting the contents of a 100 ml. graduate from each shift. I suggested that this procedure should be strengthened in two ways. The first was to obtain the weights on 100 pupae from each graduate. The second was to obtain each successive one-tenth of the graduate's contents from each one-tenth of the pupae trays put up during the shift, i.e., if 130 trays are put up during the shift, take 10 ml. from 1st tray, 10 ml. from the 14th tray, 10 ml. from the 27th tray, etc. This should be done in duplicate with two graduates being put up. Again, I concurred with a suggestion by Dr. Davis that the sampling be more intensive for two weeks time. During this time, two graduates are to be filled from the first two trays sampled, two graduates from the next two trays, etc. for a total of 10 graduates during each shift. Weights are to be taken on 100 pupae from each graduate. This intensive sampling over two weeks time should give us an idea as to whether the minimal sampling which I recommended is adequate.

The final decision over whether large flies or small flies are more desirable will have to be made by the people at Mission. If they decide that the larger flies are preferred, they can obtain them either by shifting to the HAD strain or by selecting for large pupae among the plant strain. I feel that a combination of these two procedures may be preferable. Mr. Graham is now in the process of establishing a 21-day cycle in the plant with HAD strain flies.

Attachment

cc:

Dr. Gartman, Mission, Texas



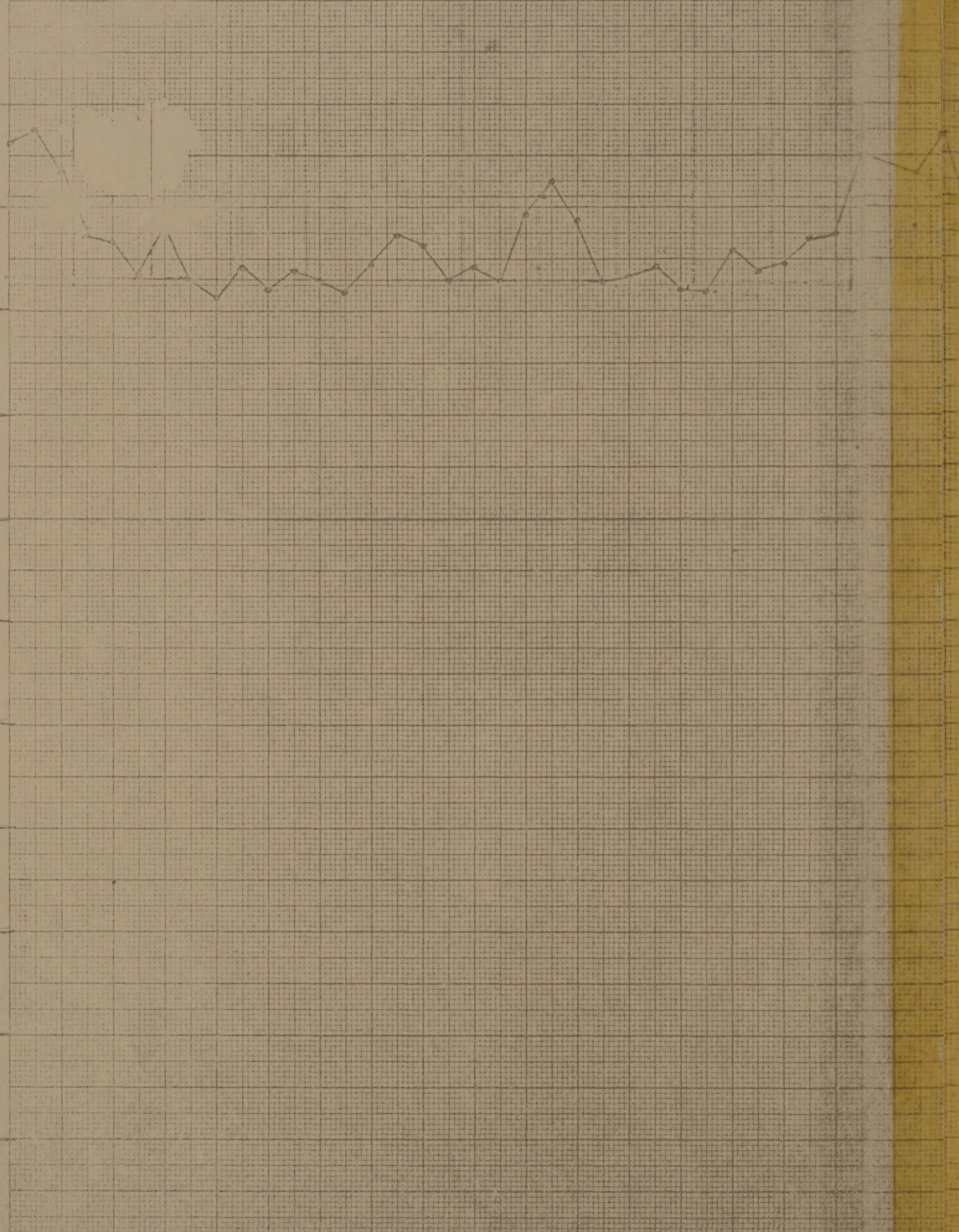
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$\frac{1}{3}$ N _u						$\frac{1}{5}$ H	$\frac{1}{5}$ S _h			

Jan Feb Mar Apr May June July Aug Sept Oct Nov Dec

Number of Larvae per 100cc.

1,100
1,000
900
800
700
600
500
400
300
200
100

1-5-63 2-5-63 3-25 5-5 6-15 7-25 9-5 10-15 11-25 1-5-64



NOTES ON HYDROPONIC DISCUSSION

February 18, 1965

10:30 A.M.

Dr. Gartman said sometime ago it was decided that to solve our hydroponic problem we would have to identify it and this we have done. The problem you are mainly concerned with, as I recall, is your small larvae, Dr. Gingrich stated. This is right. While all artificially reared screwworms are smaller than the native flies, we feel there is a reason for the small "hydro" flies since originally they were larger. We have identified two areas that could be responsible for the small size: one is the genetic factor - we may have bred up smaller worms, or it may be nutritional.

Dr. Gingrich asked if we have pinpointed - are we strongly sure it is one or the other, or could it be entirely inherent.

Dr. Gartman said we know (1) that in the past growing larvae artificially we have been able to get bigger sized worms than we are getting in our present production. (2) We also know that shortly before we went on "hydroponics" we put on a selection pressure to collect the early crawl-off in order to get a greater percentage of females in the Fly Colony and this may have attributed to the smaller size. (3) We also know that in the early work Jack Graham did with the hydroponic media in use we did get worms comparable in size to the ones on meat.

Dr. Gingrich pointed out we may not have a true picture of what is wrong if our method of measuring is not sound or statistically accurate. Dr. Gartman indicated Dr. Davis and Dr. Beal have questioned this and while our measuring techniques may not have been all they should have been, it is the same measuring technique we have always used and up until this time we haven't had a need for having a better measuring system.

Dr. Gartman further stated that on the early SAG tests Mr. Baumhover made on hydroponic flies he generally got equal performance from "hydros" and once or twice better performance.

Dr. Gingrich asked for an explanation of our method of sampling for weights. Mr. Graham advised that from the time larvae begin leaving the vat until they finish crawl-off we have collected two samples of 100 larvae every four hours and these are weighed and averaged. He said Dr. Beal felt a total of four samples should have been sufficient. Dr. Gingrich felt that with "wet" weights you may or may not get the information you want. In his own experience in nutritional work he indicated larval weights to be the only indices; however, the usual way is not to measure "wet" weight but "dry" weight since you get a lot of variables with water content which makes up about 80% of the larval body weight. He felt the only way to get a true indices of larval size would be to put our method of sampling on a more sound experimental basis and use "dry" weight as your established technique.

Mr. Graham asked for method in determining dry weight. Dr. Gingrich said you would first kill the larvae by dropping them in chloroform and then place them in a drying oven and leave them until they look dry to you. Then you would

10:30 A.M.

February 18, 1955

Dr. Gortman said sometime ago it was decided that to solve our hydraulic problem we would have to identify it and take care of it. The problem was not really concerned with, as I recall, the small larvae, Dr. Gortman stated. This is right. While all essentially correct statements are similar from the nature of the thing, we feel there is a reason for the small "hydraulic" stress originally they were larger. He have identified two areas that could be responsible for the small stress: one is the genetic factor - we may have bred up smaller worms, or it may be nutritional.

Dr. Gortman asked if we have pinpointed - are we strongly sure it is one or the other, or could it be entirely inherent.

Dr. Gortman said we know (1) that in the past growing larvae artificially we have been able to get higher sized worms than we are getting in our present production line. (2) We also know that shortly before we went on "hydraulic" we got on a selection program to collect the early over-off in order to get a greater percentage of larvae in the fly colony and this may have contributed to the smaller size. (3) We also know that in the early work Jack Gortman did with the hydraulic media in use we did get worms comparable in size to the ones on meat.

Dr. Gortman pointed out we may not have a true picture of what is wrong if our method of measuring is not sound or systematically accurate. Dr. Gortman indicated Dr. Davis and Dr. Hall have questioned this and while our measuring techniques may not have been all they should have been, it is the same measuring technique we have always used and up until this time we haven't had a need for having a better measuring system.

Dr. Gortman further stated that on the early 5th stage Mr. Buchsner made on hydraulic flies he got a fairly good equal performance from "hydraulic" and once or twice better performance.

Dr. Gortman asked for an explanation of our method of sampling for weights. Mr. Gortman advised that from the time larvae begin leaving the vat until they finish crawling he have collected two samples of 100 larvae every four hours and these are weighed and averaged. He said Dr. Hall felt a total of four samples should have been sufficient. Dr. Gortman said that with "hydraulic" you may or may not get the information you want. In his own experience in nutritional work he indicated larval weights to be the only indicator; however, the usual way is not to measure "weight" but "dry" weight since you get a lot of variation with water content which makes up about 80% of the larval body weight. He felt the only way to get a true picture of larval size would be to put out method of sampling on a more sound experimental basis and not "dry" weight as your variable technique.

Dr. Gortman asked for method in determining dry weight. Dr. Gortman said you would first fill the larvae by dropping them in alcohol and then place them in a drying oven and leave them until they look dry to you. Then you would

remove them, weigh them, and continue to weigh them at periodic intervals until there is no further change in weight - in other words, until you have a constant weight. He further felt it might be advisable to move this work from the main rearing floor into the Methods Laboratory as it is easier to work with smaller numbers. Once you get an established sound technique for sampling and measuring then you can run comparative tests with the selected strain, the ENT strain on your best solid media (horse meat) and on fluid media. If on the meat diet only your selected strain is small, then your size problem is an inherent one. If on hydroponics both come off small, then it would probably be nutritional.

Mr. Graham asked if it made any difference on the time they come off the vats; however, Dr. Gingrich indicated that when larvae have finished feeding and come off the vats on their own accord, they have stored up all the solids they are going to.

Dr. Gartman asked how long after larvae evacuate the vats does digestion and secretion take place. Mr. Graham said in sawdust about 12-16 hours.

Dr. Gartman asked if there would be any difference in the dry weight between when larvae normally come off the vats and in the pre-pupae stage, or would this pre-pupae stage be the best time for checking of weights. Dr. Gingrich felt there would not be much difference without the gut, and the amount of material remaining in the gut in solid and in fluid rearing should balance out. He felt it might be more difficult to sample the pre-pupae stage on routine plant production. However, after you have established a sound technique for sampling and determining weights, this method can be used easily and with accuracy to determine results of many different comparative tests; i.e., those discussed above, ratios between dry and wet weights, etc.

Dr. Gingrich asked several questions about the liquid diet now in use in trying to determine if in our hydroponic media we are introducing a great deal more water than used in our normal solid media. Though most of the dried products are reconstituted to the same ratio as the whole product (blood was cited as an example), we do use possibly 50 more gallons of solution to a vat. He indicated that when you add water and salt to a diet you set up osmotic imbalances and he was trying to determine whether our fluid-reared worms might have a greater water content than those reared on solid media. This possibility is another reason why "dry weights" are more accurate in the larval stage.

Dr. Gingrich further stated it is extremely important that samples be taken from the entire culture from the first to the last so that you not only get total weight but the variables (ups and downs) as well, and when you run comparative tests you must standardize all factors - constant temperatures, diet, feeding intervals, etc. or you introduce another variable, and you run into trouble if you measure more than one variable at a time.

Asked what size samples we should take Dr. Gingrich stated that in his nutritional tests he worked with very small numbers since he had to consider cost of the nutrients, labor and all, but he thought 100 a good round figure, or you could set up small test vats and get them all, since the more you get, the better.

Dr. Gartman felt this was good except for one problem which we have run into in the past - that is that when we use the small laboratory test vats we get a certain set of results but when we transfer this same set of conditions onto the rearing floor, we can't duplicate the same set of facts. Dr. Gingrich felt that if the diet is the same, then the reason for the difference must be physical factors.

Dr. Gartman asked if Dr. Gingrich has seen our hydroponic rearing as we are doing it now on mass basis, and he indicated he hadn't seen it since he was here about a year ago. Dr. Gartman stated there is some nutritional differences from when he had seen it before and Dr. Gingrich said he was planning to go into the plant this afternoon.

Mr. Graham advised that sometimes these larvae come off accidentally before they are fully grown and we get more pre-mature larvae on hydroponics than on horse meat. Dr. Gingrich said that is just "part of the game" since you are measuring what would be the weight of the entire production. He felt, however, that to get accurate statistics we should run through at least two generations. He asked if we had a balance scale. Mr. Graham stated there is one in his office but it had never been used; they have been weighing in grams.

Dr. Gartman asked if there would be any advantage in measuring dry weight of pupae? Dr. Gingrich thought not since you get into a lot more variables there as pupae are metabolized - they aren't just laying there. If you are pretty sure they are the same age you could compare them but it is much more difficult to do. I don't know just what you would have to consider in the pupal stage, except that they give off water and gas which, of course, would affect the dry weight - you have more leeway on larvae. For instance, four hours difference in pupae age would be a vast difference, whereas a larva when he finishes feeding doesn't do much of anything.

It was pointed out we may have a small pupae and a small fly but it may be just as big in weight as one that has a bigger skeleton. How well these two different bugs behave in the field may or may not be significant, but we think the bigger skeleton will have more weight to it, and that it will have more fat bodies and stored nutrients and more vigor to get the job done in the field. Dr. Gingrich thought we could assume this but he didn't know if anyone could prove this.

There was detailed discussion of the present liquid diet in use, the constituents of various dried products used and this information was furnished to Dr. Gingrich for his further study. Dr. Gingrich stated that screwworms don't need any sugars at all - in fact, they may be inhibitory - what they do need is protein and vitamins to metabolize them. He asked further if we had any idea of the chlorine content of the water we are using and Dr. Gartman stated we will find this out for him. (3-4 PPM chlorine) He also asked about the salt content and was advised that while it is quite high in some parts of the Valley, it is better here than in most areas.

Dr. McBride asked how sensitive are screwworms to amino acids. Dr. Gingrich stated that if they are too concentrated it will kill them and if the essential ones are not present development will be retarded. He indicated the ability to convert them is different and certain essentials cannot be converted, but generally all organisms require about the same amino acids. What we have to be sure of is to have the essential proteins there; it is up to the worm to break them down. Dr. McBride wondered if in the dehydration process of some products you might not get some changes that would be detrimental, and there is this possibility, of course; however, Dr. Gingrich indicated that from the preliminary review of the analyses of the various products being used in hydroponics, it appeared that we have all the necessary essentials for larval growth and development.

Dr. Hightower stated he had a question. Dr. Beal, a Statistician and Geneticist, has been down and gone over all the data and will send down a report. Let's assume first he will say the major source of our problem stems from selection which has produced genetic changes and this is $3/4$ of our problem and $1/4$ is due to some other factor. He wanted to know what service Dr. Gingrich could offer as to how we might determine whether this other part is nutritional. Dr. Gingrich stated first you would have to establish a measuring technique on the dry weight basis as we have discussed and secondly set up tests as discussed to determine if it is inherent. If you find that it is, you might just as well start over with new stock since we can't change a genetic makeup. Then we will worry about the nutritional aspect; however, as far as I can tell the nutrients we need are in the diet we are using. What I am recommending on sampling will give you a good measuring tool and may take some of the bias out in our interpretation. It won't change the size but what we want is to make sure that we are getting a good index. What Dr. Beal says from a statistical standpoint may help to determine what percent is genetic and what percent is nutritional. If it develops it is partially nutritional, all you can do is experiment with your diet and take test samples until you find the very best for growth and development.

Dr. Gartman expressed appreciation for the efforts Entomology Research has made to help us solve this problem and he felt our discussion this morning has helped to clarify a lot of things. He stated we are open to any suggestions on anything we can do that would help to solve this problem and we are grateful for the time Dr. Gingrich took from his regular research activities to come down and help us.

Meeting adjourned at 11:45 A.M.

HYDROPONIC DISCUSSION

January 21, 1965

10:40 A.M.

Dr. Gartman called the meeting to order and advised in this session we need to decide what we want to propose as a start toward solution of our hydroponic problems.

Mr. Smith proposed we start taking fly colony pupae from the line instead of selecting them since we are back to a 50-50 female-male ratio in the colony now. This would eliminate a lot of extra work. It will also mean we will get "hydro" and lung flies in our colony roughly at the same percentage we have in production which is about 80-20.

Mr. Smith said an item that should have been brought up in the staff meeting was whether or not it was thought wise to hold back a little lung or go ahead and use it all, our supply in Harlingen being approximately 150,000 pounds. Mr. Reed could see no advantage in holding any back indicating that if we decide we need lungs, we could get them in two weeks and any change from hydroponics would be preplanned sufficiently in advance for necessary procurement.

Mr. Smith further stated some of the plant foremen would like to go to all hydroponics and see what takes place.

Getting back to hydroponics, Dr. Gartman said our first suggestion proposed eliminating selection pressure to get first larvae coming off for the colony. He thought it apparent in last week's discussion that we have worked ourselves out of advantage in doing it this way. He asked if Dr. Hightower or anyone else could see a reason for continuing the selection pressure but none could. Dr. Hightower suggested we might want to speed up Mr. Graham's buildup of a colony from the ENT strain which has never been accelerated and eventually switch production to this strain to eliminate the early maturing flies.

((DECISION)) Eliminate selection pressure and go back to collecting pupae off the line for the colony. Mr. Graham will continue buildup of colony from ENT strain with decision deferred till later on whether to abandon our "early developers" in favor of the ENT colony (original Bithlo-Fla. strain).

Mr. Reed asked if previous discussion on Blood Proteins doesn't warrant our trying some tests with fresh blood as a replacement for dried blood in the fluid media to see if it makes a difference in larval size. He further felt we should run this test through several generations.

Mr. Graham stated that while we haven't made any large comparisons on fresh blood versus dried blood in the fluid media, those we did run showed no difference between the two. He also stated they didn't run more tests with fresh blood since the purpose of a hydroponic rearing process was to get away from refrigeration, and the fact that blood, especially fresh blood, is one of the most difficult of the rearing ingredients to work with because of coagulation.

There was considerable discussion on blood coagulation and whether or not it would be harmful in the liquid-rearing process. Also, whether this testing should be done on the line or in the Methods laboratory, how long it should be run to get an accurate statistical analysis of its effect on size, etc. It was determined that small vat tests will show the nutrient value between the two, and there would be no need to work out the mechanics for handling fresh blood on the rearing floor unless the small vat tests show some promise of the fresh blood being much better than the dried blood.

((DECISION)) Mr. Graham will set up a laboratory test using the ENT strain to check the nutrient value of fresh blood versus dried blood in the fluid media.

Meeting adjourned at 11:15 A.M.

Data on some physical requirements of screw-worm larvae, eg. temperature, are available and should be applicable to hydroponic rearing. It need only be emphasized that strict adherence to these requirements be observed. A new factor, the osmotic pressure of the rearing medium, may have been introduced and should be investigated.

The nutritional or chemical requirements of screw-worm larvae have been studied but due to the complexity of the problem further studies may be needed. Components of the hydroponic medium have been changed to meet technical requirements and it is likely that the best possible formulation is not now in use. However, due to the complexity of the factors involved and the fact that a large degree of success is possible with the present formulation it is advised that the present formulation be continued until other factors, such as the inherent nature of the flies, be investigated.

In addition, it was advised that all further studies be done under controlled conditions in containers smaller than the present mass rearing vats and that dry larvae weights be used to assess the results. Also, sampling techniques should be used which will yield data for statistical analysis.

The general proposals presented above are discussed further as follows:

A. Test and sampling procedures:

1. Conduct all rearing tests in small bulb heated vats that are presently available. Check thermostatic controls to insure maintenance of optimum temperatures. Hold vats in mass rearing room where they can be maintained by 24 hr. attention.
2. Seed each test with a measured volume of eggs (calibrated to deliver a predetermined number) in the range from 800-1000.
3. Collect all mature larvae that crawl from the vats in 8 hr. periods. Record number of larvae obtained for each period and randomly divide into 2 equal groups. Place one-half in pupating medium for breeding stock, combine pupae obtained from each 8 hr. period into a single breeding stock. Place remaining half of larvae from each period in chloroform, remove when dead and transfer **to pre-weighed, uncovered petri dish.** Place dead larvae in oven (80°C) and dry until constant weight is achieved -- record total weight and divide by number of larvae to get mean weight of individual larvae for entire test. In addition, randomly select and make individual weights (to nearest $1/10\text{ mg}$) of 20% of larvae in each group. Total individual weights and obtain mean weights of individual larvae for each time period. Analyze individual larvae weight data to determine if significant difference occurs between

data on some physical requirements of stress-free larvae, etc. Temperature, was recorded and should be applicable to hydroponic feeding. It was only as suggested that slight adjustment to these requirements be observed. A new set of the specific pressure of the feeding medium, may have been introduced and should be investigated.

The nutritional or chemical requirements of stress-free larvae have been studied but due to the complexity of the problem (various studies may be needed). Components of the growth medium have been changed to meet certain requirements and it is likely that the best possible formulation is not now in use. However, due to the complexity of the factors involved and the fact that a large degree of success is possible with the present formulation it is advised that the present formulation be continued until other factors, such as the indicated factors at the time, be investigated.

In addition, it was advised that all further studies be done under controlled conditions in containers smaller than the present ones, feeding rate and that any larvae which are used to assess the results. Also, sampling techniques should be used which will yield data for statistical analysis.

The general progress presented above was discussed further as follows:

A. Test and sampling procedures:

1. Conduct all feeding tests in small bell shaped vials that are constantly available. Check temperature controls in larvae maintenance of optimum temperature. Hold vials in case feeding from which they can be subjected to 10 hr. attention.
2. Feed each test with a measured volume of eggs (calculated to collect a predetermined number) in the range from 500-1000.
3. Collect all mature larvae that travel from the vials in 5 hr. periods. Record number of larvae collected for each period and randomly divide into 1 equal groups. Place one-half in petting medium for breeding stock. Remove pupae obtained from each 5 hr. period into a sterile breeding stock. Place remaining half of larvae from each period in incubator, remove when dead and transfer to pre-weighed, uncovered petri dish. Place dead larvae in oven 100° C and dry until constant weight is achieved -- record total weight and divide by number of larvae to get mean weight of individual larvae for each test. In addition, randomly select and mark individual weights for analysis (1/2 wt. of 1/2 - 1 larvae in each group). Total individual weights and record mean weights of 100 larvae for each time period. Also, 100 individual larvae weight data to determine if significant difference exists between

have to be repeated. It is advised that future studies on other variables in larvae rearing be followed by thorough studies with the adults.

8. Studies on the nutritive qualities of the various materials used in hydroponic studies should be deferred until the data has been analyzed from the genetic studies above. Methods and Developments Section of ADE presently has on hand samples of various nutritive materials which should be tested. Such tests, if done, should be carried out under the same precisely controlled conditions described for the genetic tests. Larval dry weight data should be obtained and analyzed and also studies on the adult vitality would be necessary.

Plum felt

FORMULA FOR LIQUID MEDIUM

To make up 1 liter of medium: (1000 ml.)

60 grams dried blood

30 grams dried suckle milk

30 grams dried egg

30 grams dried cottage cheese

2 ml. formalin

850 ml. water

Mix all ingredients well. Add the same quantity of horsemeat as for usual starting medium (500 grams horsemeat:500 ml. fluid). Reduce horsemeat to 200 grams:800 ml. fluid if medium is too thick.

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Mission

June 4, 1965

To: O. H. Graham
Investigations Leader
Livestock Insects Investigations
Kerrville, Texas

From: B. G. Hightower
Entomologist in Charge

Subject: Laboratory evaluation of male screw-worm flies reared on liquid medium

The sizes, dry residual weights, and mating abilities of male screw-worm flies reared on liquid medium, horse meat, and wounded sheep have been compared in a series of laboratory tests.

All size and weight measurements were made on lots of 10 unfed male flies which had eclosed within a 2-hour time interval from the same lots of flies used in the mating tests. Sizes are given in arbitrary units based on the product of 2 linear measurements. The ether-soluble fats were extracted, weighed, and subtracted from the dry weights to obtain the dry residual weights. Observed matings among 5-day old virgin male and female flies were recorded for 1 hour in each test. Each cage contained 3 males from the test strain and 30 animal-reared females from the Mexican strain.

In the first 6 tests, comparisons were made with flies from the strain used by ADE since the initiation of the Southwestern Program. In the remaining tests, flies were used from an ENT strain which was sub-cultured from the ADE strain in 1961. The mean matings per male in the first 6 tests were as follows:

Mexican Animal-reared Unirradiated	Mexican Animal-reared Irradiated	Liquid-reared Irradiated	Horse Meat- reared Irra- diated
1.5	1.5	2.9	4.9

The infrequency of matings by recently colonized strains of flies has been noted previously. However, the mean difference between the horse meat-reared males and the remaining males was above the 1% level of significance by Duncan's Multiple Range Test. Similar results were obtained with the ENT strain of flies.

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Mission

June 4, 1962

To: O. H. Graham
Investigations Leader
Livestock Insects Investigations
Kerrville, Texas

From: E. G. Highower
Entomologist in Charge

Subject: Laboratory evaluation of male screw-worm flies reared on lipid medium

The sizes, dry residual weights, and mating abilities of male screw-worm flies reared on lipid medium, horse manure, and wounded sheep have been compared in a series of laboratory tests.

All size and weight measurements were made on lots of 10 unfed male flies which had eclosed within a 2-hour time interval from the same lot of flies used in the mating tests. Sizes are given in arbitrary units based on the product of 2 linear measurements. The ether-soluble fats were extracted, weighed, and subtracted from the dry weights to obtain the dry residual weights. Observed matings among 5-day old virgin males and females were recorded for 1 hour in each test. Each cage contained 3 males from the test strain and 30 animal-reared females from the Mexican strain.

In the first 6 tests, comparisons were made with flies from the strain used by ADE since the initiation of the Southern Program. In the remaining tests, flies were used from an EHT strain which was sub-cultured from the ADE strain in 1961. The mean matings per male in the first 6 tests were as follows:

Mexican Animal-reared	Mexican Animal-reared	Lipid-reared	Horse Manure-reared
Unfed	Irradiated	Irradiated	Irradiated
1.2	1.2	2.2	4.2

The infrequency of matings by recently colonized strains of flies has been noted previously. However, the mean difference between the horse manure-reared males and the remaining males was above the 1% level of significance by Duncan's Multiple Range Test. Similar results were obtained with the EHT strain of flies.

The mean size of the male flies over all 14 tests are given below:

<u>Mexican Animal-</u> <u>reared</u>	<u>Liquid-</u> <u>reared</u>	<u>Horse Meat-</u> <u>reared</u>
522.2 units	308.9 units	418.4 units

These differences were quite obvious and varied little among the different lots of males.

The dry residual weights of lots of 10 males from each of the 14 tests were as follows:

<u>Mexican Animal-</u> <u>reared</u>	<u>Liquid-</u> <u>reared</u>	<u>Horse Meat-</u> <u>reared</u>
15.2 mg./fly	7.2 mg./fly	11.3 mg./fly

These differences were also consistent among the lots.

In summary, flies reared on liquid medium are smaller and weigh less than flies reared from wounds or on horse meat medium. These differences affect the ability of the males to successfully mate with wound-reared females. Whether or not this disadvantage can be overcome in the field by releasing larger numbers of irradiated males per unit area is highly speculative and should be thoroughly tested.

The mean size of the male flies over all 14 tests are given below:

Tested	Tested	Tested
322.3 units	308.9 units	418.4 units
Mexican Animal-	Liquid-	Horse Meat-

These differences were quite obvious and varied little among the different lots of males.

The dry residual weights of lots of 10 males from each of the 14 tests were as follows:

Tested	Tested	Tested
12.2 mg./fly	7.2 mg./fly	11.3 mg./fly
Mexican Animal-	Liquid-	Horse Meat-

These differences were also consistent among the lots.

In summary, flies reared on liquid medium are smaller and weigh less than flies reared from wounds or on horse meat medium. These differences affect the ability of the males to successfully mate with wound-reared females. Whether or not this disadvantage can be overcome in the field by releasing larger numbers of irradiated males per unit area is highly speculative and should be thoroughly tested.

In the first 5 tests, comparisons were made with flies from the control used by the state the initiation of the tsetse fly program. In the remaining tests, flies were used from an area which was not infested from the tsetse fly in 1941. The mean weights per male in the 14 tests were as follows:

Tested	Tested	Tested
1.3	1.3	1.3
Mexican Animal-	Liquid-	Horse Meat-

The relationship of weight to fecundity is not known at this time. In the first 5 tests, the mean difference between the control and the irradiated males was about 10% in favor of the irradiated males. In the remaining tests, the difference was about 5% in favor of the irradiated males.

Mr. Graham

JUL 8 1965

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ANIMAL DISEASE ERADICATION DIVISION
FEDERAL CENTER BUILDING
HYATTSVILLE, MARYLAND 20781

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To : E. F. Knipling
Director
ERD, ARS, Beltsville, Maryland

JUL 7 1965

From : Donald Miller
Acting Director

Subject: The mating potential of liquid media-reared screwworm flies

We, too, have been concerned with the small size of the screwworm larvae reared on a liquid media. We have closely followed the tests that are being conducted on their mating abilities in the laboratory, and we also question their ability to effectively compete with the larger animal-reared native flies under field conditions. We expect to obtain information on their field competitiveness from the Poza Rica and the Sinaloa field tests.

We do not believe that all of our problems with the outbreaks that are occurring in the United States arise from the non-competitiveness of the small fly. Each year we have anticipated that flies from outside the treated area will penetrate the barrier. The past winter and spring have been ideal for the propagation of the screwworm fly. Unusually mild weather has created an environment in the Southwest that has placed maximum stress upon the barrier and even with the best of flies, it is believed that there would have been similar scattered outbreaks.

Tests conducted at Mission indicate the liquid media may be deficient in some nutritional factors, although larvae now being produced are larger than those observed by you during your recent visit to Mission. The size of the larvae now compares favorably with those reared on lung or whale meat. Even though larger larvae are now being produced, we have decided to phase back into the use of solid media until more information can be developed as to the cause of the smaller flies. Initially, our plans are to place about 25 percent of our production on solid media. We probably will use horse meat. In the meantime, we intend to make every effort to improve the liquid diet.

We would appreciate further discussions with you on screwworm diets.

Donald Miller

JUL 3 1965

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ANIMAL DISEASE ERADICATION DIVISION
FEDERAL CENTER BUILDING
HYATTSVILLE, MARYLAND 20781

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JUL 7 1965

To : E. F. Knippling
Director
ERD, ARS, Beltsville, Maryland

From : Donald Miller
Acting Director

Subject: The mating potential of lipid media-reared screwworm flies

We, too, have been concerned with the small size of the screwworm larvae reared on a lipid media. We have closely followed the tests that are being conducted on their mating abilities in the laboratory, and we also question their ability to effectively compete with the larger animal-reared native flies under field conditions. We expect to obtain information on their field competitiveness from the Pora Rica and the Sinaloa field tests.

We do not believe that all of our problems with the outbreaks that are occurring in the United States arise from the non-competitiveness of the small fly. Each year we have anticipated that flies from outside the treated area will penetrate the barrier. The past winter and spring have been ideal for the propagation of the screw worm fly. Unusually mild weather has created an environment in the Southwest that has placed maximum stress upon the barrier and even with the best of flies, it is believed that there would have been similar scattered outbreaks.

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Donald Miller

Dr. Hightower

JUN 21 1965

June 16, 1965

To: F. J. Mulhern, Director, Animal Disease Eradication Division

From: E. F. Knippling, Director, Entomology Research Division

Subject: The mating potential of liquid media-reared screw-worm flies

During my visit to Mission when the special program for the Secretary was held, I had occasion to discuss in some detail with our research staff the quality of the screw-worm flies currently released in carrying out the screw-worm control program. I also had the opportunity to go through the rearing plant to see the procedures used and to note the size of the larvae, pupae, and adults. The small size of the flies that are being produced is a matter of great concern to me and to members of our research staff. In the discussions we had, it was brought out by Dr. Hightower that his laboratory evaluations suggest that the liquid-reared flies are not equally competitive with the horse meat-reared irradiated flies. Unfortunately, we are not sure what laboratory results mean in terms of actual performance under field conditions. However, in the absence of field data, I believe that we are forced to place considerable significance in results of laboratory evaluations as an indication of the relative performance of different types of flies that are produced.

In replicated tests, the findings of the laboratory tests indicate that liquid-reared irradiated flies mated an average of 2.9 times with Mexican animal-reared females in one hour periods, as compared with an average of 4.9 times for irradiated males that were reared on horse meat. This would indicate a substantial difference in the capacity for mating for the two types of reared and irradiated males when exposed to large females reared on animals, which would be representative of wild females. To provide some correlation with size of the insects used, it might be mentioned that the mean size of the three kinds of flies were as follows: Mexican animal-reared males, 500.22 units; horse meat media-reared males, 418.4 units; and liquid media-reared males, 308.9 units. On a dry residual weight basis, the relative weights were as follows: Mexican animal-reared males, 15.2 mg. per fly; horse meat-reared males, 11.3 mg. per fly; and liquid media-reared males, 7.2 mg. per fly. The small flies reared on the liquid medium may be inferior from a physiological standpoint, but in addition, observations indicate that the small males are handicapped in mating with large animal-reared females because of their small size.

We recognize that plans are under development to conduct tests under field

conditions. However, we all realize the difficulty in making close evaluations between fly strains under the many variable conditions one encounters in the field. It seems to us that serious consideration should be given to producing at least a substantial percent of the flies by the regular rearing procedure until we have sufficient information to show that the insects reared by the current method are equal in quality to the type of flies employed by the older rearing method. We recognize the great economic advantage of the liquid media rearing method. This is an important development. At the same time, the possibility exists that we could expect equal or greater efficiency from releases of fewer flies that are reared by methods that have been demonstrated to be highly effective in previous operations.

cc:

Mr. McDuffie
Dr. Graham
Dr. Hightower
Dr. Bushland

C. F. Kripling

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 flies by the rearing procedure until we have sufficient infor-
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 quality to the type of flies employed by the other rearing method. We
 recognize the great economic advantage of the hybrid rearing method.
 This is an important development. At the same time, the possibility
 exists that we could expect equal or greater efficiency from releases of
 lower flies that are reared by methods that have been demonstrated to be
 slightly effective in previous operations.

L. E. Knight

cc:
 Mr. McNeill
 Dr. Graham
 Dr. Hightower
 Dr. Bushland



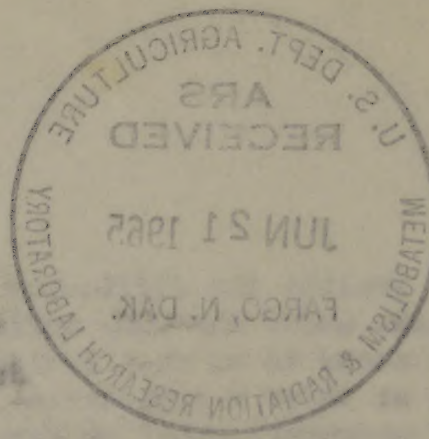
June 16, 1965

To: F. J. Mulhern, Director, Animal Disease Eradication Division
From: E. F. Knipling, Director, Entomology Research Division
Subject: The mating potential of liquid media-reared screw-worm flies

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In replicated tests, the findings of the laboratory tests indicate that liquid-reared irradiated flies mated an average of 2.8 times with Mexican animal-reared females in one hour periods, as compared with an average of 4.9 times for irradiated males that were reared on horse meat. This would indicate a substantial difference in the capacity for mating for the two types of reared and irradiated males when exposed to large females reared on animals, which would be representative of wild females. To provide some correlation with size of the insects used, it might be mentioned that the mean size of the three kinds of flies were as follows: Mexican animal-reared males, 500.22 units; horse meat media-reared males, 418.4 units; and liquid media-reared males, 308.9 units. On a dry residual weight basis, the relative weights were as follows: Mexican animal-reared males, 15.2 mg. per fly; horse meat-reared males, 11.3 mg. per fly; and liquid media-reared males, 7.2 mg. per fly. The small flies reared on the liquid medium may be inferior from a physiological standpoint, but in addition, observations indicate that the small males are handicapped in mating with large animal-reared females because of their small size.

We recognize that plans are under development to conduct tests under field



To: F. J. Milner, Director, Animal Disease Research Division
From: E. F. Knippling, Director, Entomology Research Division
Subject: The nesting potential of lipid-treated screwworm flies

During my visit to Mexico when the special program for the Secretary was held, I had occasion to discuss in some detail with our research staff the quality of the screw-worm flies currently released in carrying out the screw-worm control program. I also had the opportunity to go through the nesting plant to see the procedures used and to note the size of the larvae, pupae, and adults. The small size of the flies that are being produced is a matter of great concern to me and to members of our research staff. In the discussion we had, it was brought out by Dr. Knippling that his laboratory evaluations suggest that the lipid-treated flies are not equally competitive with the horse meat-treated irradiated flies. Unfortunately, we are not sure what laboratory results mean in terms of actual performance under field conditions. However, in the absence of field data, I believe that we are forced to place considerable significance in results of laboratory evaluations as an indication of the relative performance of different types of flies that are produced.

In replicated tests, the findings of the laboratory tests indicate that lipid-treated irradiated flies mated an average of 3.8 times with Mexican animal-treated females in one hour periods, as compared with an average of 4.9 times for irradiated males that were reared on horse meat. This would indicate a substantial difference in the capacity for mating for the two types of treated and irradiated males when exposed to large females reared on animals, which would be representative of wild females. To provide some correlation with size of the insects used, it might be mentioned that the mean size of the three kinds of flies were as follows: Mexican animal-treated males, 580.22 mg.; horse meat-treated males, 418.4 mg.; and lipid media-treated males, 508.9 mg. On a dry weight basis, the relative weights were as follows: Mexican animal-treated males, 15.3 mg. per fly; horse meat-treated males, 11.3 mg. per fly; and lipid media-treated males, 7.5 mg. per fly. The small flies reared on the lipid medium may be inferior from a physiological standpoint, but in addition, observations indicate that the small males are handicapped in mating with large animal-treated females because of their small size.

It is recognized that plans are under development to conduct tests under field

conditions. However, we all realize the difficulty in making close evaluations between fly strains under the many variable conditions one encounters in the field. It seems to us that serious consideration should be given to producing at least a substantial percent of the flies by the regular rearing procedure until we have sufficient information to show that the insects reared by the current method are equal in quality to the type of flies employed by the older rearing method. We recognize the great economic advantage of the liquid media rearing method. This is an important development. At the same time, the possibility exists that we could expect equal or greater efficiency from releases of fewer flies that are reared by methods that have been demonstrated to be highly effective in previous operations.

cc:

Mr. McDuffie
Dr. Graham
Dr. Hightower
Dr. Bushland

E. J. Kripling

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cc:
Mr. McDuffie
Mr. Graham
Dr. Hightower
Dr. Bushland

E. J. Burdick

Mr. Ashland

JUL 8 1965

TECH

AMV
JFC
RRR

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ANIMAL DISEASE ERADICATION DIVISION
FEDERAL CENTER BUILDING
HYATTSVILLE, MARYLAND 20781

To : E. F. Knipling
Director
ERD, ARS, Beltsville, Maryland

JUL 7 1965

From : Donald Miller
Acting Director

Subject: The mating potential of liquid media-reared screwworm flies

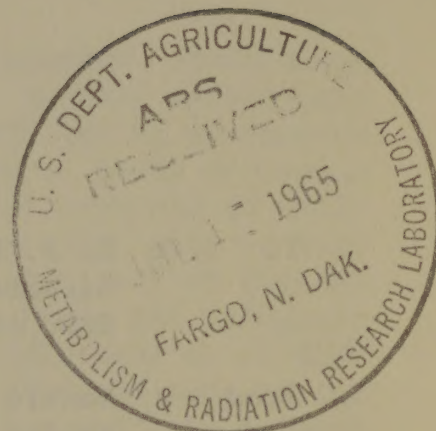
We, too, have been concerned with the small size of the screwworm larvae reared on a liquid media. We have closely followed the tests that are being conducted on their mating abilities in the laboratory, and we also question their ability to effectively compete with the larger animal-reared native flies under field conditions. We expect to obtain information on their field competitiveness from the Poza Rica and the Sinaloa field tests.

We do not believe that all of our problems with the outbreaks that are occurring in the United States arise from the non-competitiveness of the small fly. Each year we have anticipated that flies from outside the treated area will penetrate the barrier. The past winter and spring have been ideal for the propagation of the screw-worm fly. Unusually mild weather has created an environment in the Southwest that has placed maximum stress upon the barrier and even with the best of flies, it is believed that there would have been similar scattered outbreaks.

Tests conducted at Mission indicate the liquid media may be deficient in some nutritional factors, although larvae now being produced are larger than those observed by you during your recent visit to Mission. The size of the larvae now compares favorably with those reared on lung or whale meat. Even though larger larvae are now being produced, we have decided to phase back into the use of solid media until more information can be developed as to the cause of the smaller flies. Initially, our plans are to place about 25 percent of our production on solid media. We probably will use horse meat. In the meantime, we intend to make every effort to improve the liquid diet.

We would appreciate further discussions with you on screwworm diets.

Donald Miller



Screwworm

February 28, 1966

To: W. C. McDuffie, Chief, Insects Affecting Man and
Animals Research Branch, ENT, ARS, USDA
Plant Industry Station
Beltsville, Maryland 20705

From: R. C. Bushland, Laboratory Director

Subject: Screwworm fly quality and screwworm field tests in
Mexico in 1966

This concerns your letter of February 11 to Dr. Graham with carbon copies to Dr. Hightower and me. I am sending copies of this memorandum to Drs. Knipling, Graham, and Hightower. In your memorandum of February 11 you suggested that Dr. Graham should tell the program people that Dr. Knipling favored all meat rearing until hydroponic flies were proved equal.

This subject came up at the joint conference last July. I believe Dr. Hightower was present for the discussion, but I think Dr. Graham had already left the meeting for a trip to Mexico. The ANH people brought out that just a month or so earlier they had purchased a whole year's supply of ingredients for liquid rearing, using the funds available at the end of the preceding fiscal year. They also emphasized that the budget for the current fiscal year was extremely tight and that switching to all meat rearing would be extremely difficult from the budget viewpoint.

Dr. Knipling said that in view of those considerations he would go along with half liquid-reared and half meat-reared flies, providing that the production was mixed so that both types of flies were released together.

The the December 15 conference, Dr. Mulhern again raised the question about hydroponic flies. I think that he brought up this point because we had earlier discussed the poor performance of hydroponic

February 28, 1966

W. C. McNeill, Chief, Insects Affecting Man and
Animal Research Branch, EHT, ARS, USDA
Plant Industry Station
Beltsville, Maryland 20705

From: R. C. Bushland, Laboratory Director

Subject: Screwworm fly quality and screwworm field tests in
Mexico in 1966

This concerns your letter of February 11 to Dr. Graham with carbon
copies to Dr. Rightower and me. I am sending copies of this memo-
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of February 11 you suggested that Dr. Graham should tell the program
people that Dr. Knippling favored all meat testing until hydropenic
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funds available at the end of the preceding fiscal year. They also
emphasized that the budget for the current fiscal year was extremely
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go along with half lipid-tested and half meat-tested flies,
providing that the production was mixed so that both types of flies
were released together.

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Page 6
W. C. McDuffie
February 28, 1966

flies in the Poza Rica field test. Being mindful of Dr. Knippling's comments last July, and knowing that the budget was still tight, I said that our Division still favored 50-50 production with the flies mixed before release. This was reported on pages 8 and 9 of the minutes of the December 15 conference.

Since ANH does not have enough money to switch over completely to horse meat rearing and the ingredients for hydroponic rearing are already bought and paid for, I see no harm in putting out the 50-50 mixture of flies. This costs very little more than cutting in half the fly rearing and releasing horse meat flies only at half the present rates over the same flight lanes.

cc: E. F. Knippling
O. H. Graham
B. G. Hightower

cc of McDuffie's 2 letters of Feb-11 to Mr. Graham
enclosed with Mr. Knippling's copy.

cc of Mr. Bushland's memo of Feb-16 enclosed with
cc of this memo to Graham + Hightower.

flies in the Ford Rice field test. Being mindful of Mr. Knippling's comments last July, and knowing that the budget was still tight, I said that our Division still favored 50-50 production with the flies mixed before release. This was reported on pages 8 and 9 of the minutes of the December 12 conference.

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cc: E. F. Knippling
O. H. Graham
B. C. Righwater

FEB 28 1966

R.C. Bushland

February 11, 1966

To: O. H. Graham, Kerrville

From: W. C. McDuffie, Chief, Insects Affecting Man and Animals
Research Branch

Subject: Report of Poza Rica Test

Reference is made to your letter of January 13 transmitting Dr. Davis' report on the subject test and your analysis of the results.

From my point of view it is very difficult to make any definite conclusion from the results of the test. The first part strongly suggested that the meat reared flies were superior but we need similar results after the shift to really tie this down. Unfortunately, weather conditions and other factors apparently wrecked any chances of confirmation.

I referred the entire record to Dr. Knipling and here are his comments.

"This is enough to convince me that we should raise the best flies possible. Also, we need to consider possible changes in behavior and should not use too wide swaths for releases. There is a big difference between 17% and 6% in a program even though statistically, it may be difficult to show significant differences."

W.C. McDuffie

cc:
B.G. Hightower
R.C. Bushland

R. C. Bushland

FEB 28 1966

February 11, 1966

To: O. K. Graham, Kerrville
From: W. C. McWhorter, Chief, Insects Affecting Man and Animals
Research Branch
Subject: Report of Foxe Rice Test

Reference is made to your letter of January 13 transmitting Dr. Davis' report on the subject test and your analysis of the results.

From my point of view it is very difficult to make any definite conclusion from the results of the test. The first part strongly suggested that the most reared flies were superior but we need similar results after the shift to really be this done. Unfortunately, weather conditions and other factors apparently worked any chances of confirmation.

I referred the entire record to Dr. Knippling and here are his comments.

"This is enough to convince me that we should raise the best flies possible. Also, we need to consider possible changes in behavior and should not use too wide swaths for releases. There is a big difference between 1% and 5% in a program even though statistically, it may be difficult to show significant differences."

W. C. McWhorter

cc: B. G. Hightower
R. C. Bushland

R.C. Bushland

February 11, 1966

To: O. H. Graham, Kerrville

From: W. C. McDuffie, Chief, Insects Affecting Man and Animals
Research Branch

Subject: Screw-worm fly quality and screw-worm field tests in
Mexico in 1966

Reference is made to your two letters of January 20 on the above subjects. These were referred to Dr. Knipling for his comments. A copy of his comments are attached and one is also being sent to Dr. Hightower.

Dr. Knipling's letter clearly indicates his concern over the quality of flies being released. However, I have also discussed this matter with him and can assure you that his letter does not really reflect his much deeper concern in this matter. He would like to know just what your feeling on the matter is and what if anything is being done at the program level to improve fly quality. I told him I did not know for sure what the program people were doing but I believed they were rearing about half of the flies on liquid media and half on meat. It is obvious from the 3rd paragraph of his letter that he favors all meat rearing until such time as the efficiency of the liquid media-reared flies have been shown to be equal to that of meat reared flies.

This is a rather delicate matter to discuss with the program people but you may think it wise to do so. In any event we would like information on the current situation and what might be projected for the future.

We really do not feel competent to comment on the occurrence of mutants in the field larval samples. However, we feel as you do that the matter needs to be investigated along the lines you mentioned. In this connection, I would like to know just what procedures are now followed in the quality control section. At one time I believe this activity was dropped or virtually so and then resumed. I have no information at all on current procedures, but I feel strongly that quality control is highly important and should be a continuing activity.

Enclosure
cc: (w/cy encl)
R.C. Bushland
B.G. Hightower

W. C. McDuffie

February 11, 1956

To: O. W. Graham, Kerrville
From: W. C. McButtle, Chief, Insects Affecting Man and Animals
Research Branch
Subject: Screw-worm fly quality and screw-worm field tests in
Mexico in 1955

Reference is made to your two letters of January 20 on the above
subjects. These were referred to Dr. Knippling for his comments.
A copy of his comments are attached and one is also being sent to
Dr. Hightower.

Dr. Knippling's letter clearly indicates his concern over the quality
of flies being released. However, I have also discussed this matter
with him and can assure you that his letter does not really reflect
his much deeper concern in this matter. He would like to know just
what your feeling on the matter is and what if anything is being
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activity was dropped or virtually so and then resumed. I have no
information at all on current procedures, but I feel strongly that
quality control is highly important and should be a continuing activity.

W. C. McButtle

Enclosure
cc: (W.C. and)
R. C. Bushland
B. S. Hightower

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ENTOMOLOGY RESEARCH DIVISION
BELTSVILLE, MARYLAND 20705

Insects Affecting
Man & Animals

FEB 10 1966

W.C.M.
D.E.W.
J.A.F.

February 9, 1966

To: W. C. McDuffie, Branch Chief, Insects Affecting Man and
Animals Research Branch

From: E. F. Knipling, Director, Entomology Research Division

Subject: Rearing screw-worms for release

Dr Graham's memo of January 20 to you on the subject of screw-worm field tests in Mexico in 1966, contained one statement that is disturbing to me. The statement indicates that the liquid-media reared flies are regarded as being equal to the horsemeat-reared flies and that it would be feasible to go back to a 100% liquid-medium operation.

As expected before the field test in Mexico was conducted, it was extremely difficult to prove differences in performance of released flies because of the many variable factors encountered in such operations. However, from the data I saw several months ago I certainly would not conclude that the tests showed the liquid reared flies to be equal in efficiency to the horsemeat flies. Unless there is additional information that I have not seen to show that the liquid flies are equal in performance to horsemeat-reared flies, I would definitely oppose the use of liquid-media reared flies of the type that have been reared in the past.

I am fearful that the attitude might prevail that liquid-media reared flies should be used unless it can be shown that horsemeat-reared flies are better. This is not my view. I feel strongly that horsemeat-reared flies should be reared until it can be shown that liquid-media reared flies are equal to the horsemeat-reared flies. In other words, I think the burden of proof should be on the liquid-media reared flies before they are adopted as the standard fly for release.

Obviously, we are all interested in producing flies as economically as possible. However, I think the quality of the fly released is the most important consideration. We have several years background experience with the horsemeat-reared insects. The population was brought under control with flies reared in this manner. The laboratory observations, the apparent slow response to control of isolated populations last year, and the trend to less efficiency in the Poza Rica test, plus the basic judgment that stunted animals of any kind are inferior all point to one conclusion on my part. The conclusion that it would be a great mistake to release only liquid-media reared flies unless it is shown that these flies are equal or superior to those of the type reared before.

If there is recent information that justifies a change in the opinion I expressed last year, I would very much like to have a report on it.

E. J. Snipe

February 16, 1966

To: E. F. Knipling, Director
Entomology Research Division, ARS, USDA
Plant Industry Station
Beltsville, Maryland 20705

From: R. C. Bushland, Laboratory Director

Subject: Rearing screwworms for release

Thank you for the copy of your memorandum of February 9 on the above subject.

I have not seen a copy of Dr. Graham's memorandum of January 20 to which you refer, but on the basis of my conversation with him in late December I think that he is opposed to hydroponic flies.

I am attaching a thermofax copy giving excerpts from pages 8 and 9 of Summary of Minutes of Joint Technical Planning Conference, December 15, 1965. You will note that the ideas which Dr. Graham and I expressed, as summarized in the report of that conference, are in complete agreement with the thoughts in your letter of February 9.

I think that the ANH officials are in complete agreement that liquid-reared flies will not be used to replace horsemeat-reared flies until they are proved equal in field tests. I think that it will be a very long time before such proof is obtained.

I note from the first paragraph on page 2 of the Summary of Minutes of Staff Conference of January 13 that the Sinaloa field test is to start soon. In that test they will try (1) 12-mile swaths, (2) large release cartons, and (3) a comparison of liquid- and horsemeat-reared flies. Last December I cautioned the Mission veterinarians against trying three new things all at one time. I also told them that if in one field test hydroponic flies should perform as well as horsemeat-reared flies, I would accept those results only to apply to that particular area under the conditions of weather prevailing at the season when that test was made. I said that I did not assume that the results could be applied to other localities, or even to the same locality, at another season of the year.

From the attitude shown by Dr. Mulhern and Dr. Anderson, I think you will find those officials just as conservative as you are regarding reliance on horsemeat-reared flies.

Enclosure

cc:

W. C. McDuffie

Enclosure

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Subject:

Rearing screwworms for release

From:

R. C. Bushland, Laboratory Director

To:

E. F. Knippling, Director
Entomology Research Division, ARS, USDA
Plant Industry Station
Beltsville, Maryland 20705

February 16, 1966

Thank you for the copy of your memorandum of February 9 on the above subject.

Mr. Bushland

FEB 15 1966

February 9, 1966

To: W. C. McDuffie, Branch Chief, Insects Affecting Man and
Animals Research Branch

From: E. F. Knipling, Director, Entomology Research Division

Subject: Rearing screw-worms for release

Dr Graham's memo of January 20 to you on the subject of screw-worm field tests in Mexico in 1966, contained one statement that is disturbing to me. The statement indicates that the liquid-media reared flies are regarded as being equal to the horsemeat-reared flies and that it would be feasible to go back to a 100% liquid-medium operation.

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Obviously, we are all interested in producing flies as economically as possible. However, I think the quality of the fly released is the most important consideration. We have several years background experience with the horsemeat-reared insects. The population was brought under control with flies reared in this manner. The laboratory observations, the apparent slow response to control of isolated populations last year, and the trend to less efficiency in the Poza Rica test, plus the basic judgment that stunted animals of any kind are inferior all point to one conclusion on my part. The conclusion that it would be a great mistake to release only liquid-media reared flies unless it is shown that these flies are equal or superior to those of the type reared before.

FEB 12 1966

February 9, 1966

To: J. E. Whittie, Branch Chief, Insect Attracting Unit and
Antenna Research Branch

From: E. F. Kuhlman, Director, Entomology Research Division

Subject: Antenna Research - 1965 release

On January 13, 1966, I was on the subject of antenna research. The antenna is a complex structure that is distributed in the body of the insect. The antenna is composed of many segments, each of which has a specific function. The segments are arranged in a linear fashion, and each segment is connected to the next by a joint. The segments are of varying lengths, and the joints are of varying degrees of flexibility. The antenna is a highly sensitive organ, and it is able to detect a wide range of stimuli, including light, sound, and chemical signals. The antenna is also able to sense the position and movement of the insect's head and body. The antenna is a complex organ, and it is a subject of ongoing research.

An excellent feature of the field test in Mexico was the extensive use of the antenna. The antenna is a complex structure that is distributed in the body of the insect. The antenna is composed of many segments, each of which has a specific function. The segments are arranged in a linear fashion, and each segment is connected to the next by a joint. The segments are of varying lengths, and the joints are of varying degrees of flexibility. The antenna is a highly sensitive organ, and it is able to detect a wide range of stimuli, including light, sound, and chemical signals. The antenna is also able to sense the position and movement of the insect's head and body. The antenna is a complex organ, and it is a subject of ongoing research.

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If there is recent information that justifies a change in the opinion I expressed last year, I would very much like to have a report on it.

cc:

Dr. R. C. Bushland ✓

ARS:ENT:EFKnipling:eaj

REFERENCE SLIP

4/5/66

TO

Dr. W. G. Lawrence

mm

- | | |
|--|---|
| ☐ ACTION | ☐ NOTE AND RETURN |
| ☐ APPROVAL | ☐ PER PHONE CALL |
| ☐ AS REQUESTED | ☐ RECOMMENDATION |
| ☐ FOR COMMENT | ☐ REPLY FOR SIGNATURE OF |
| ☐ FOR INFORMATION | ☐ RETURNED |
| ☐ INITIALS | ☐ SEE ME |
| ☐ NOTE AND FILE | ☐ YOUR SIGNATURE |

REMARKS

For your info. Copy
Sent to Hogg.

mas

Our estimate

FROM

DATE

1944

1944

- | | |
|--|---|
| ☐ 1. Name | ☐ 2. Address |
| ☐ 3. City | ☐ 4. State |
| ☐ 5. Zip | ☐ 6. Telephone |
| ☐ 7. Occupation | ☐ 8. Education |
| ☐ 9. Marital Status | ☐ 10. Number of Children |
| ☐ 11. Date of Birth | ☐ 12. Sex |
| ☐ 13. Race | ☐ 14. Religion |
| ☐ 15. Political Party | ☐ 16. Other |

1944

April 4, 1966

To: R. C. Bushland, Director, Metabolism & Radiation Research
Laboratory, Fargo, North Dakota

From: E. F. Knipling, Director, ENT

Subject: Screw-worm rearing procedures

Thank you for your memorandum of March 25. I am sure we all appreciate the time and thought you have given to this matter. I agree with your views regarding hydroponic-reared flies. I went along with the 50-50 ratio of regular lean meat-hydroponic flies at the meeting you referred to because of available supplies for hydroponic rearing. However, I would recommend against the purchase of additional material for rearing hydroponic flies (except for producing flies for experimental purposes).

I will state my views, which correspond to yours, as follows: I recommend that all screw-worm flies used for releases in the program be reared on meat in accordance with the rearing procedure used before hydroponic flies were produced, employing any modifications that may produce flies equivalent in size. The number of flies released per unit area should be reduced if necessary to stay within the budget for rearing flies. I would not sacrifice the size of the area where flies need to be dropped but rather reduce the number dropped, particularly in areas of lowest natural population density.

To the extent that research and control workers feel justified, I would continue to evaluate hydroponic-reared flies, but if the same expenditure on improved rearing methods offer possibilities, this might be a more productive procedure to follow. At any rate, I would not change to hydroponic flies unless field tests under typical ecological conditions show the two types of flies to be essentially equal in performance.

My reasons for questioning the justification for employing hydroponic flies are as follows:

1. Laboratory observations suggest that small flies may be less competitive than larger flies in mating with large wild females because of size alone.

2. The established self-generated populations last season did not seem to be brought under control as quickly in 1965 as they were in 1964.

3. The meat-reared flies have a history of success under a wide range of conditions over a period of 5 years or so. I don't think we should take the risk of a major change in rearing procedure without good evidence to show that the flies are equal or superior.

4. The field tests conducted are not conclusive one way or the other but based on data I saw, the trend seemed to indicate that the hydroponic flies were inferior.

I think we need to continue research on more economical procedures for rearing flies or for producing better quality flies at comparable costs. This is one of the important advances that can be made. However, my thinking always has been to try to develop flies that are larger even than the horse meat flies. The size and general appearance of media-reared flies, even when horse meat is used, indicate some nutritional deficiency. I have always assumed that media-reared flies comparable in size and shape to wild flies would be more competitive and more adapted to the environment.

ARS:ENT:EPKnipling:av

C. F. Knipping

APR 7 1966

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ENTOMOLOGY RESEARCH DIVISION
BELTSVILLE, MARYLAND 20705

April 4, 1966

To: R. C. Bushland, Director, Metabolism & Radiation Research
Laboratory, Fargo, North Dakota

From: E. F. Knipling, Director, ENT

Subject: Screw-worm rearing procedures

Thank you for your memorandum of March 25. I am sure we all appreciate the time and thought you have given to this matter. I agree with your views regarding hydroponic-reared flies. I went along with the 50-50 ratio of regular lean meat-hydroponic flies at the meeting you referred to because of available supplies for hydroponic rearing. However, I would recommend against the purchase of additional material for rearing hydroponic flies (except for producing flies for experimental purposes).

I will state my views, which correspond to yours, as follows: I recommend that all screw-worm flies used for releases in the program be reared on meat in accordance with the rearing procedure used before hydroponic flies were produced, employing any modifications that may produce flies equivalent in size. The number of flies released per unit area should be reduced if necessary to stay within the budget for rearing flies. I would not sacrifice the size of the area where flies need to be dropped but rather reduce the number dropped, particularly in areas of lowest natural population density.

To the extent that research and control workers feel justified, I would continue to evaluate hydroponic-reared flies, but if the same expenditure on improved rearing methods offer possibilities, this might be a more productive procedure to follow. At any rate, I would not change to hydroponic flies unless field tests under typical ecological conditions show the two types of flies to be essentially equal in performance.

My reasons for questioning the justification for employing hydroponic flies are as follows:

1. Laboratory observations suggest that small flies may be less competitive than larger flies in mating with large wild females because of size alone.

2. The established self-generated populations last season did not seem to be brought under control as quickly in 1965 as they were in 1964.

3. The meat-reared flies have a history of success under a wide range of conditions over a period of 5 years or so. I don't think we should take the risk of a major change in rearing procedure without good evidence to show that the flies are equal or superior.

4. The field tests conducted are not conclusive one way or the other but based on data I saw, the trend seemed to indicate that the hydroponic flies were inferior.

I think we need to continue research on more economical procedures for rearing flies or for producing better quality flies at comparable costs. This is one of the important advances that can be made. However, my thinking always has been to try to develop flies that are larger even than the horse meat flies. The size and general appearance of media-reared flies, even when horse meat is used, indicate some nutritional deficiency. I have always assumed that media-reared flies comparable in size and shape to wild flies would be more competitive and more adapted to the environment.

C. J. Snippling

March 25, 1966

To: E. V. Knippling, Director
Entomology Research Division, ARS, USDA
Plant Industry Station
Beltsville, Maryland 20705

From: R. C. Bushland, Director

Subject: Screwworm rearing procedures

Enclosed is a Thermofax copy of the first two pages of minutes of the March 17 Screwworm Staff Conference. I thought that you would be particularly interested in the paragraph beginning near the bottom of page one and ending on page two.

In your memorandum of February 9 to Mr. McDuffie, subject "Rearing screw-worms for release," you expressed concern that screwworm production might be switched to hydroponic rearing on the basis of an opinion expressed by Dr. Graham in a memorandum of January 20. I think that this March 17 report should reassure us all that the ARS group will not make such a change.

Dr. Fitcher, who is now Acting in Charge at Mission, told me that the next quarterly technical meeting would be held around the 10th to 15th of April and that I would be invited as soon as the date was definite. I expect that at that meeting the subject of procurement of additional hydroponic supplies will arise and that my opinion on such procurement will be requested.

If you concur, I would like to tell the group that I am against ordering any more hydroponic supplies for routine production. If the Mission plant runs out of hydroponic supplies and needs additional quantities to complete some field trials, I would go along with field test quantities.

I had the feeling last summer when you agreed to 50-50 production of hydroponic and horse-meat flies, that you did so only because the hydroponic materials were on hand, and that this year's budget did not permit a complete switchover to horse meat. However, for next fiscal year, hydroponic supplies would have to be bought with funds

Page 2
E. F. Enipling
March 25, 1966

that could otherwise go for horse meat or nutria, and I think that it would be better to rely entirely on red meat even if it involved some curtailment in numbers of flies produced.

If the Sinaloa test now underway should produce results that look favorable to the hydroponic flies, there might be some pressure to accept those data as proof of the equality of hydroponic flies. My own feeling is that the Sinaloa test cannot be considered conclusive, and that favorable results there could only justify further field trials of hydroponic flies but would not warrant switching from horse meat to hydroponics or going back to 50-50 production now that the hydroponic supplies have been exhausted.

Would you please advise me whether or not you agree.

Enclosure

cc:

W. C. McHaffie (with Thermofax sheets)
G. B. Graham

RCB:hbp

March 25, 1966

To: E. F. Knipling, Director
Entomology Research Division, ARS, USDA
Plant Industry Station
Beltsville, Maryland 20705

From: R. C. Bushland, Director

Subject: Screwworm rearing procedures

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I had the feeling last summer when you agreed to 50-50 production of hydroponic and horse-meat flies, that you did so only because the hydroponic materials were on hand, and that this year's budget did not permit a complete switchover to horse meat. However, for next fiscal year, hydroponic supplies would have to be bought with funds

March 22, 1966

E. F. Knapling, Director
Entomology Research Division, ARS, USDA
Plant Industry Station
Beltsville, Maryland 20705

To:

R. C. Bushland, Director

From:

Scientific testing procedures

Subject:

Enclosed is a photostatic copy of the first two pages of minutes of the March 17 Entomology Staff Conference. I thought that you would be particularly interested in the paragraph beginning near the bottom of page one and ending on page two.

In your memorandum of February 9 to Mr. McCallie, subject "Starting water-worms for release," you expressed concern that hydroponic production might be switched to hydroponic testing on the basis of an opinion expressed by Dr. Graham in a memorandum of January 20. I think that this March 17 report should reassure us all that the ANH group will not make such a change.

Dr. Fischer, who is now Acting in Charge at Mission, told me that the next quarterly technical meeting would be held around the 10th to 15th of April and that I would be invited as soon as the date was definite. I expect that at that meeting the subject of procurement of additional hydroponic supplies will arise and that my opinion on such procurement will be requested.

If you concur, I would like to tell the group that I am against ordering any more hydroponic supplies for routine production. If the Mission plant runs out of hydroponic supplies and needs additional quantities to complete some field trials, I would go along with field test quantities.

I had the feeling last summer when you agreed to 20-25 production of hydroponic and horse-meat flies, that you did so only because the hydroponic materials were on hand, and that this year's budget did not permit a complete switchover to horse meat. However, for next fiscal year, hydroponic supplies would have to be bought with funds

Page 2
E. F. Knipling
March 25, 1966

that could otherwise go for horse meat or nutria, and I think that it would be better to rely entirely on red meat even if it involved some curtailment in numbers of flies produced.

If the Sinaloa test now underway should produce results that look favorable to the hydroponic flies, there might be some pressure to accept those data as proof of the equality of hydroponic flies. My own feeling is that the Sinaloa test cannot be considered conclusive, and that favorable results there could only justify further field trials of hydroponic flies but would not warrant switching from horse meat to hydroponics or going back to 50-50 production now that the hydroponic supplies have been exhausted.

Would you please advise me whether or not you agree.

Enclosure

cc:

W. C. McDuffie (with Thermofax sheets)
O. H. Graham

RCB:hop

Page 2
H. V. Knippling
March 23, 1966

that could otherwise go for horse meat or mutton, and I think that it would be better to rely entirely on red meat even if it involved some curtailment in numbers of flies produced.

If the flies that now undergo should produce results that look favorable to the hydroponic flies, there might be some pressure to accept those data as proof of the equality of hydroponic flies. My own feeling is that the flies that cannot be considered conclusive, and that favorable results there could only justify further field trials of hydroponic flies but would not warrant switching from horse meat to hydroponics or going back to 50-50 production now that the hydroponic supplies have been exhausted.

Would you please advise me whether or not you agree.

Enclosure

cc:

W. C. McBride (with Theriot's abstract)
O. N. Graham

RCB:hob

Dr. Bushland
B
Entomology Research Division
Kerrville, Texas

Conference on screw-worm nutrition

JUL 25 1966

W. C. McDuffie, Chief
Insects Affecting Man and
Animals Research Branch
Beltsville, Maryland

July 21, 1966

Although their studies are far from complete, Gingrich, Hightower, and A. J. Graham have already obtained some very interesting preliminary information concerning screw-worm nutrition. These data very strongly indicate that larval development is seriously interrupted by transferring the larvae from starting vats to finishing vats at 36 hours of age. In two tests, they have obtained heavier prepupae by placing newly hatched larvae on the test diet rather than transferring started larvae to the test diet. Dr. Gingrich now feels that an abrupt change of diet may be a shock to the larvae, due to the differences in the digestive enzymes required for the starting diet and the finishing diet.

In one test which is now being replicated, pork lung appeared to be equal to a liquid medium containing 10% egg and slightly superior to the standard horsemeat diet. If further tests support this finding, this information alone will be of considerable practical value to the program. Because of the high cholesterol content of both egg and lung, Dr. Gingrich feels that the presence of cholesterol in quantity may explain the much heavier wet weight of prepupae from those diets. He has suggested that it would perhaps be an improvement in the rearing procedure to start the larvae on a low-cholesterol diet and gradually increase the cholesterol content so that the larvae would be finished on the high-cholesterol diet. Two weeks from now more data will be available from tests now in progress or outlined for next week.

I have proposed to Dr. Pitcher that we have a joint staff meeting of ENT and ANH Division personnel on August 4 to discuss the implications of the information obtained on nutrition so far. It is too early to make a definite recommendation for changed rearing procedures, but I feel that group discussion would be helpful in planning additional research as well as minor modifications in ANH rearing procedures. It would also be an appropriate time to discuss ways of using the Mexican strain of screw-worm flies. I suggested to Dr. Pitcher that Dr. Bushland be invited to attend

this meeting if he can arrange the travel. It would also be very helpful if you and Dr. Knipling could take part in these discussions. I am not proposing a regular Quarterly Technical Conference, but merely a slight expansion of the regular weekly staff meeting which is held at Mission on Thursdays.

O. H. Graham

O. H. Graham
Investigations Leader
Livestock Insects Investigations

In duplicate

cc:

R. C. Bushland ✓

S. C. Gartman

B. G. Hightower ✓

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proposing a regular General Technical Conference, but merely a slight
expansion of the regular weekly staff meeting which is held at Mission
on Thursdays.

W. G. Highower

G. H. Graham
Investigator
Livestock Research Investigation

In duplicate

- ✓ G. H. Graham
- ✓ R. C. Carlson
- ✓ W. G. Highower

Mr. Bushland

FEB 20 1967

Entomology Research Division
Kerrville, Texas

Screw-worm larval diets

S. C. Cartman
Assistant Veterinarian in Charge
of Screw-Worm Eradication Activities
Animal Health Division
Mission, Texas

February 16, 1967

After an absence of several weeks from my office, I have read with a great deal of interest the accumulated reports of the Weekly Staff Meetings and other reports on the screw-worm program. I was especially interested in the discussion of the tests with larval diets and rearing problems. We all realized at the time they were initiated, I believe, that the tests jointly planned and conducted by Gingrich and A. J. Graham would be time-consuming, but I must admit that I am disappointed with the progress of these studies. It is not surprising that program officials have looked for outside means of intensifying the Methods Development effort.

I do not have any information concerning the contacts that have been made with Batelle Institute except for that in the reports of Weekly Staff Meetings, so I do not know just who approached whom or what plans there may be for paying for this outside research contract. Actually the Methods Development and Entomology Research support for the entire Southwestern Eradication Program has been extremely limited, and I believe that all of us have been unhappy with the diminution of this effort as compared to the much better support for the Southeastern program. In my personal opinion, many of the problems which have arisen in connection with the Southwestern program could have been avoided had the combination of Methods Development and Entomology Research activities been able to respond more rapidly to the needs of the program. So, if outside research support can be obtained, we will certainly welcome any help which can be obtained. However, if it is planned to pay for this research with appropriated funds of the Department of Agriculture, I think that a careful comparison should be made of the cost of conducting this research outside the Government with in-house research. In general, research conducted by private laboratories is quite expensive, and I would expect this to be true in the case of Batelle Institute since, as far as I know, they do not have anyone on their staff with specialized experience in insect nutrition or screw-worm rearing.

I might point out that some of the inconclusive results which have been obtained in the tests conducted so far by Gingrich and Graham have been

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due to lack of proper laboratory facilities and equipment. In some of the recent tests, the temperature of the trays in the lower parts of the improvised rearing cabinet dropped below 60°F. This is only one of the problems connected with attempting to conduct tests of this type with inadequate equipment and laboratory technician support. In other words, I feel that if there are any funds available for supplementing the Methods Development effort, consideration should be given to using this money for purchase of well designed rearing cabinets, minimal laboratory facilities, and for hiring competent laboratory technicians to carry out the tests. It would perhaps be desirable to add professional scientists to your Methods Development staff also if the funds are available.

As Dr. Hightower has pointed out in your staff meetings and as I have mentioned in prior correspondence (see my memorandum of November 16, 1965, to you), there is very strong evidence to indicate that the rearing problems in the plant are at least as much mechanical in nature as nutritional. It would be a serious mistake, I believe, to build a new screw-worm rearing plant in Mexico which merely duplicated the Mission plant. The rearing trays at Mission were designed for a solid larval medium, and it is not surprising that problems have arisen when a liquid medium was used in the same trays. For this, and a number of other problems, it would be desirable to add a competent engineer with entomological experience and an entomologist with insect physiology training to your Methods Development section. It seems to me that money invested in this way would be much more likely to produce solutions to the problems of fly production for the Southwestern and Mexico programs than the same money in outside research contracts.

One of the problems in studying improved diets is choosing between empirical and basic approaches to the research problems. While fundamental studies of screw-worm nutrition such as amino acid conversion, enzyme activity, symbiotic relationships, etc., etc., would be very interesting, they are unlikely to solve your relatively short-range practical problems. An empirical approach by which various combinations of economical materials are tested by actually rearing screw-worms in them appears to be preferable. And as you have said many times, we have more "know-how" on screw-worm rearing within USDA than exists anywhere else in the world.

I am pleased to note that you called a Technical Conference for March 9. Perhaps it would be appropriate to set aside a substantial block of time for a thorough discussion of both the Methods Development and Entomology Research programs and how these can be improved. Solution of the rearing problems is very important, and it is especially urgent that procedures

due to lack of proper laboratory facilities and equipment. In some of the recent tests, the temperature of the trays in the lower parts of the incubated rooms varied below 50°F. This is only one of the problems connected with attempting to conduct tests of this type with inadequate equipment and laboratory facilities support. In other words, I feel that if there are any funds available for supplementing the methods development effort, consideration should be given to using this money for purchase of well designed testing equipment, minimal laboratory facilities, and for hiring competent laboratory technicians to carry out the tests. It would perhaps be desirable to add professional scientists to your methods development staff also if the funds are available.

As Dr. Hightower has pointed out in your staff meeting and as I have mentioned in prior correspondence (see my memorandum of November 10, 1951, to you), there is very strong evidence to indicate that the testing problems in the plant are at least as much mechanical in nature as physiological. It would be a serious mistake, I believe, to build a new serum-worm testing plant in Mexico which merely duplicated the Mexican plant. The testing trays at Mexico were designed for a solid larval medium, and it is not surprising that problems have arisen when a liquid medium was used in the same trays. For this, and a number of other problems, it would be desirable to add a competent engineer with entomological experience and an entomologist with insect physiology training to your Methods Development section. It seems to me that money invested in this way would be much more likely to produce solutions to the problem of fly production for the Southwestern and Mexican programs than the same money in outside research contracts.

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especially adapted for the use of the available nutrient materials be developed before a new plant and its equipment are designed.

O. H. Graham

O. H. Graham
Investigations Leader
Livestock Insects Investigations

cc:

D. E. Weidhaas (2)
B. G. Hightower
R. C. Bushland ✓
R. S. Sharman

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G. E. Graham
Investigation Leader
Livestock Investigation

G. E. Weidner (2)
D. G. Hightower
J. C. Goodland
E. E. Searson

File

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ENTOMOLOGY RESEARCH DIVISION
BELTSVILLE, MARYLAND 20705

June 6, 1967

To: Dr. D. E. Weidhaas, Room 124, North Building, Plant
Industry Station, Beltsville, Maryland 20705

From: W. E. Robbins, Principal Insect Physiologist and
S. R. Dutky, Research Biologist

Subject: Consultation - screw-worm rearing at Mission, Texas

At the suggestion of Dr. Hugh Graham, we are sending this summary of our observations and recommendations concerning the development of an improved liquid medium for screw-worm rearing to be used when the program moves into Mexico. The following comments are a composite of those mutually arrived at through discussions with Dr. Gingrich and Mr. Jack Graham, and through examining the data from the carefully planned exploratory experiments that Mr. Jack Graham and Dr. Gingrich have carried out. The results of their tests show that a basal medium consisting of dried blood (6%) and Suckle (4%) resulted in larvae which weighed only 0.3 - 0.4 mg each at the end of 48 hours and which were not of sufficient size at maturity. When this basal medium was supplemented with a number of different nutritive materials, there resulted increased growth rates, larger larvae at the end of 48 hours and larvae whose final weight was as great as or greater than those from the currently used blood-meat diet. The major objection given to the use of certain of these supplements was their cost. Based on the available experimental evidence our comments, suggestions, and recommendations are as follows:

- (1) Examination of the data from the above-mentioned tests indicates that the nutritional quality of the medium for the first 48 hours is very important to the final weight attained by the insect. Adding whole dry egg solids at concentrations of 1, 2, and 3% gave a direct linear response in relation to the 48-hour weight of the larvae (2.0, 3.9, and 5.2 mg respectively). However, the effect of increasing the percentage of the egg seems to be less important to the final weight of the insect (i.e. 2 and 3% dry egg solids resulted in larvae with approximately the same final weight). Based on these data, it is suggested that the dry whole egg be tested at higher concentrations to determine that concentration that gives maximum growth at 48 hours. If concentrations greater than that found to give maximum response are not inhibitory, it is recommended that an excess be used in

the diet to insure optimum nutrition during this critical period. Since growth during the initial 48 hours would only account for at most about 10% of the final weight of the larva and since the dry egg solids might only need be used during this period, the objection to the use of this supplement because of the cost would then in part be negated.

- (2) On the basis of these findings, it might then be desirable to divide the nutritional regime into two phases (as is currently being done).

(A) First 48 hours; use whole dry egg supplemented medium in excess of that found to attain maximum weight. This would be the least expensive phase in relation to medium consumption.

(B) Final phase; use a low (1%) or no egg supplementation of the diet. However, examination of the test data indicates that the basal medium requires protein supplementation. Dry defatted milk, which is a component of the Suckle, increases the final weight of insects that have been provided a diet supplemented with dry whole egg solids for the first 48 hours. Perhaps an increase in the concentration of Suckle will provide the protein that is required for this phase if the presence of carbohydrates in the Suckle are not inhibitory. It might also be worthwhile to try substituting plant hydrolysates for some of the more expensive protein sources.

- (3) Yeast extract was also found to stimulate growth during the first 48 hours. Yeast extract then, should be tested in combination with dry whole egg to determine whether or not these supplements stimulate growth because of the presence of different nutritional factors. If yeast extract stimulates when used in combination with dry egg solids (or even if it doesn't) then try a commercial B complex (water soluble) vitamin preparation in the diet during the first 48 hours. Concentrations of certain of the B vitamins may be marginal or dependent upon the particular microflora present.

- (4) Additional tests should be run to determine if the activity present in dry whole egg solids is in the yolk or in the white of the egg. It would be interesting and desirable to know the active components in the egg, but this is not necessary for the successful development of a diet. Some indication of the nature of the components may come from the nutritional tests on the egg yolk and the egg white. There may be a number of active components. For that matter, egg albumen has been shown to be a very good protein source for insects which is often better

utilized than casein.

- (5) A number of tests have been run in which an attempt has been made to supplement the diet with cholesterol and no definite growth stimulation was obtained. We have taken samples of the Suckle, the dry blood, and the dry whole egg solids and we are currently analyzing these for the cholesterol content which should tell us whether or not the concentration of this essential nutrient is adequate in the basal diet.
- (6) Based on Dr. Gingrich's previous studies on the aseptic culture of the screw-worm on a defined medium, RNA should also be tested in both the basal medium and the egg supplemented basal medium.

In our visit to the Mission screw-worm plant, several additional points worthy of investigation were brought to our attention:

- (1) From the publications on mass screw-worm rearing and from the data on yield of larvae in the current production at Mission, a utilization conversion (weight of insects reared per weight of animal tissue used) of about 11.2 percent was calculated. This is quite low in comparison to the 52 percent conversion that we have obtained with other diptera (house flies, blow flies and flesh flies) under aseptic conditions. This may indicate that the screw-worms are in intense competition for nutrient by microorganisms even in the present rearing techniques. The large amounts of ammonia released from the rearing medium may also be due to microorganisms. If a liquid medium is substituted for the current semi-solid animal tissue medium, the importance of this competition may be increased. It might be advantageous to make the liquid medium semi-solid by heat coagulating the proteins or adding a solidifying gel, and thus permitting large scale use in direct substitution for the current semi-solid animal tissue media in present procedure.
- (2) Large amounts of ammonia are given off from the media, particularly in the starting room. This could indicate that considerable quantities of valuable protein are being lost and that microflora rather than screw-worm is largely responsible for this loss. Determination of the magnitude of this loss could be made by analyzing a sample of the air in the room for ammonia and multiplying this value by the air exchange rate for the room.
- (3) Some information as to the part played by microorganisms should be obtained by a bacteriological examination of the rearing medium at different stages of the rearing process.
- (4) Analysis of the spent medium for its protein and amino acid content.

If large amounts of protein or amino acid nitrogen are residual in the spent medium, perhaps some modification of the spent medium could be made to prolong its acceptability as a substrate.

- (5) Exploratory tests should be made on a small laboratory scale.
 - (A) Aseptic culture of screw-worms on the liver and egg medium used in aseptic culture of other diptera to ascertain whether this medium would give a similar high yield of screw-worms.
 - (B) To determine whether prefermenting medium with a pure culture of Bacillus subtilis strain 10 R would stabilize the medium bacteriologically and be satisfactory for screw-worms. As this organism can grow on media containing inorganic nitrogen, it may be possible to upgrade low cost non-protein media into proteins and/or amino acids required for screw-worm nutrients.
- (6) Some additional attention might be directed towards making certain that the released insects are properly synchronized with those in the field. Under current procedures the larvae are reared in continuous light and puparia and emerging adults for rearing stock are held in continuous dark prior to eggging. These insects may be free-running and non-synchronized, and it may take some time for them to become synchronized to the field light and dark regimen after release. A skeletal photophase may be satisfactory to obtain synchronization. The large number of cages of dark adapted flies in the emergence room at the rearing plant should offer an excellent opportunity to study the response of adult screw-worms to light of different wave lengths. This item and item (5) above have already been discussed with Dr. Hightower at Mission and we have agreed to cooperate with him on these studies.

It would appear from Mr. Jack Graham's and Dr. Gingrich's experimentation that the development of a liquid diet as good as or better than the blood-meat diet for screw-worm rearing is feasible. We were informed that there is always some difficulty encountered in taking a new diet to the floor. This, however, would appear to be more of a problem in handling a new diet and in familiarizing the staff with its special requirements rather than a problem in insect nutrition.

We would like to express our gratitude to Dr. Hightower and his staff and to the various staff members of the Animal Health Division for their hospitality and the time and effort spent in familiarizing us with the program.

cc:
R. C. Bushland
C. H. Hoffmann
P. W. Oman

JUN 12 1967

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ENTOMOLOGY RESEARCH DIVISION
BELTSVILLE, MARYLAND 20705

June 6, 1967

To: Dr. D. E. Weidhaas, Room 124, North Building, Plant
Industry Station, Beltsville, Maryland 20705

From: W. E. Robbins, Principal Insect Physiologist and
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- (1) Examination of the data from the above-mentioned tests indicates that the nutritional quality of the medium for the first 48 hours is very important to the final weight attained by the insect. Adding whole dry egg solids at concentrations of 1, 2, and 3% gave a direct linear response in relation to the 48-hour weight of the larvae (2.0, 3.9, and 5.2 mg respectively). However, the effect of increasing the percentage of the egg seems to be less important to the final weight of the insect (i.e. 2 and 3% dry egg solids resulted in larvae with approximately the same final weight). Based on these data, it is suggested that the dry whole egg be tested at higher concentrations to determine that concentration that gives maximum growth at 48 hours. If concentrations greater than that found to give maximum response are not inhibitory, it is recommended that an excess be used in

the diet to insure optimum nutrition during this critical period. Since growth during the initial 48 hours would only account for at most about 10% of the final weight of the larva and since the dry egg solids might only need be used during this period, the objection to the use of this supplement because of the cost would then in part be negated.

- (2) On the basis of these findings, it might then be desirable to divide the nutritional regime into two phases (as is currently being done).
 - (A) First 48 hours; use whole dry egg supplemented medium in excess of that found to attain maximum weight. This would be the least expensive phase in relation to medium consumption.
 - (B) Final phase; use a low (1%) or no egg supplementation of the diet. However, examination of the test data indicates that the basal medium requires protein supplementation. Dry defatted milk, which is a component of the Suckle, increases the final weight of insects that have been provided a diet supplemented with dry whole egg solids for the first 48 hours. Perhaps an increase in the concentration of Suckle will provide the protein that is required for this phase if the presence of carbohydrates in the Suckle are not inhibitory. It might also be worthwhile to try substituting plant hydrolysates for some of the more expensive protein sources.
- (3) Yeast extract was also found to stimulate growth during the first 48 hours. Yeast extract then, should be tested in combination with dry whole egg to determine whether or not these supplements stimulate growth because of the presence of different nutritional factors. If yeast extract stimulates when used in combination with dry egg solids (or even if it doesn't) then try a commercial B complex (water soluble) vitamin preparation in the diet during the first 48 hours. Concentrations of certain of the B vitamins may be marginal or dependent upon the particular microflora present.
- (4) Additional tests should be run to determine if the activity present in dry whole egg solids is in the yolk or in the white of the egg. It would be interesting and desirable to know the active components in the egg, but this is not necessary for the successful development of a diet. Some indication of the nature of the components may come from the nutritional tests on the egg yolk and the egg white. There may be a number of active components. For that matter, egg albumen has been shown to be a very good protein source for insects which is often better

utilized than casein.

- (5) A number of tests have been run in which an attempt has been made to supplement the diet with cholesterol and no definite growth stimulation was obtained. We have taken samples of the Suckle, the dry blood, and the dry whole egg solids and we are currently analyzing these for the cholesterol content which should tell us whether or not the concentration of this essential nutrient is adequate in the basal diet.
- (6) Based on Dr. Gingrich's previous studies on the aseptic culture of the screw-worm on a defined medium, RNA should also be tested in both the basal medium and the egg supplemented basal medium.

In our visit to the Mission screw-worm plant, several additional points worthy of investigation were brought to our attention:

- (1) From the publications on mass screw-worm rearing and from the data on yield of larvae in the current production at Mission, a utilization conversion (weight of insects reared per weight of animal tissue used) of about 11.2 percent was calculated. This is quite low in comparison to the 52 percent conversion that we have obtained with other diptera (house flies, blow flies and flesh flies) under aseptic conditions. This may indicate that the screw-worms are in intense competition for nutrient by microorganisms even in the present rearing techniques. The large amounts of ammonia released from the rearing medium may also be due to microorganisms. If a liquid medium is substituted for the current semi-solid animal tissue medium, the importance of this competition may be increased. It might be advantageous to make the liquid medium semi-solid by heat coagulating the proteins or adding a solidifying gel, and thus permitting large scale use in direct substitution for the current semi-solid animal tissue media in present procedure.
- (2) Large amounts of ammonia are given off from the media, particularly in the starting room. This could indicate that considerable quantities of valuable protein are being lost and that microflora rather than screw-worm is largely responsible for this loss. Determination of the magnitude of this loss could be made by analyzing a sample of the air in the room for ammonia and multiplying this value by the air exchange rate for the room.
- (3) Some information as to the part played by microorganisms should be obtained by a bacteriological examination of the rearing medium at different stages of the rearing process.
- (4) Analysis of the spent medium for its protein and amino acid content.

If large amounts of protein or amino acid nitrogen are residual in the spent medium, perhaps some modification of the spent medium could be made to prolong its acceptability as a substrate.

- (5) Exploratory tests should be made on a small laboratory scale.
- (A) Aseptic culture of screw-worms on the liver and egg medium used in aseptic culture of other diptera to ascertain whether this medium would give a similar high yield of screw-worms.
 - (B) To determine whether prefermenting medium with a pure culture of Bacillus subtilis strain 10 R would stabilize the medium bacteriologically and be satisfactory for screw-worms. As this organism can grow on media containing inorganic nitrogen, it may be possible to upgrade low cost non-protein media into proteins and/or amino acids required for screw-worm nutrients.
- (6) Some additional attention might be directed towards making certain that the released insects are properly synchronized with those in the field. Under current procedures the larvae are reared in continuous light and puparia and emerging adults for rearing stock are held in continuous dark prior to egging. These insects may be free-running and non-synchronized, and it may take some time for them to become synchronized to the field light and dark regimen after release. A skeletal photophase may be satisfactory to obtain synchronization. The large number of cages of dark adapted flies in the emergence room at the rearing plant should offer an excellent opportunity to study the response of adult screw-worms to light of different wave lengths. This item and item (5) above have already been discussed with Dr. Hightower at Mission and we have agreed to cooperate with him on these studies.

It would appear from Mr. Jack Graham's and Dr. Gingrich's experimentation that the development of a liquid diet as good as or better than the blood-meat diet for screw-worm rearing is feasible. We were informed that there is always some difficulty encountered in taking a new diet to the floor. This, however, would appear to be more of a problem in handling a new diet and in familiarizing the staff with its special requirements rather than a problem in insect nutrition.

We would like to express our gratitude to Dr. Hightower and his staff and to the various staff members of the Animal Health Division for their hospitality and the time and effort spent in familiarizing us with the program.

William E. Robbins

cc:

✓ R. C. Bushland
C. H. Hoffmann
P. W. Oman

Screw worm
USDA - MRS
RECEIVED

AUG 19 1968

FARGO, N. DAK.
M & R RES. LAB.

Aug. 15, 1968

TO: Claude H. Samidt

THRU: O. H. Gram

~~FROM:~~ B. G. Hightower

SUBJECT: Field evaluation of screwworm flies research on liquid medium

The enclosed report is about as timely as last year's headlines, but it was requested at the last Joint Technical Conference.

It is my recommendation that we regard flies reared on liquid medium with reservations as to their suitability for extensive use until a more adequate field test can be conducted.

Copies of this report have been sent to Dr. Gartman.

Incl.

cc

R. C. Bishland ✓

RECEIVED
AUG 13 1968

BARCO, V. LAK.
M & R RES. LAB.

Aug 13 1968

TO: Mr. J. H. Smith

FROM: Mr. J. H. Smith

SUBJECT: [illegible]

RE: [illegible]

The enclosed report is being submitted to you as part of the [illegible] project. It is requested that you review the report and advise me of any [illegible] comments.

It is requested that you review the report and advise me of any [illegible] comments. It is also requested that you advise me of any [illegible] comments.

Copies of this report have been sent to Mr. [illegible]

Very truly,
[illegible]

J. H. Smith

Field Evaluation of Screwworm Flies Research on Liquid Medium

This field evaluation of screwworm flies reared on liquid medium was undertaken as the result of a decision made at the Joint Technical Conference on Screwworm Eradication held at Mission, Texas on May 2, 1968. The objective of this investigation was to determine if there were detectable differences in the dispersal or local distribution of irradiated flies reared on liquid medium or on ground meat medium.

PROCEDURE

Eleven releases of irradiated liquid-reared and meat-reared flies have been made in 2 trapping areas near Mission, Texas. About 15 liters of each kind of pupae were used for each of the first 2 releases. In the last 9 releases, about 10 liters of each group were used for each release. All pupae received about 7,000 r. of gamma radiation at 5½ days of age. Irradiated pupae were treated with fluorescent or acetone-soluble dye and placed in large release boxes for subsequent ground release. Each box contained about 2200 pupae.

Percentages of eclosion at the time of release ranged from 42% to 98% with a mean of 84% in the meat-reared group compared with 72% to 96% with a mean of 88% in the liquid reared group. These percentages do not represent total eclosion, but the percentages of flies that had eclosed at the time of release. There was no indication that the method of rearing affected eclosion.

In one trapping area (Panal Ranch), traps were placed near stock-tanks or in mesquite brush at distances ranging from 1 to 2.5 miles from the release point. In the second trapping area, traps were placed along the Rio Grande River at specified intervals up to 20 miles from the release point. The traps were baited with beef liver in the usual manner, and all traps were emptied daily except during inclement weather.

RESULTS

Trap catches from 6 releases on Panal Ranch were as follows:

Distance from

RP and Location

Total Flies Recaptured (♀♀)

of Trap

Meat-reared

Liquid-reared

1 Mile:

1. Mesquite	924	873
2. Mesquite	1815	1899
3. Stock tank	<u>365</u>	<u>276</u>
TOTAL	3104 (51%)	3048 (49%)

1.5 Mile:

1. Stock tank	2956	1741
2. " "	500	345
3. " "	308	211
4. " "	<u>1561</u>	<u>1481</u>
TOTAL	5325 (58.4%)	3778 (41.5%)

2.5 Mile:

1. Mesquite	786	439
2. Stock tank	<u>216</u>	<u>162</u>
TOTAL	1002 (62.5%)	601 (37.5%)

Trap catches from 5 releases on the Rio Grande River were as follows:

Trap No.	<u>a/</u>	Distance from RP (Miles)	Total Flies Recaptured (♀♀)	
			Meat-reared	Liquid-reared
1	(E&W)	5	107	40
2	(E&W)	10	20	19
3	(W)	15	5	13
4	(W)	20	<u>0</u>	<u>2</u>
			132	74

a/ Includes paired traps east and west of the release point.

Previous tests with genetically marked flies have indicated that acetone-soluble red and black dyes are retained in detectable quantities by about 90% of the treated flies. In these same tests, fluorescent orange was less effective than fluorescent green. Thus, the figures in the above totals include catches from releases in which about equal numbers of both groups of flies were treated with each fluorescent dye. Since the acetone-soluble blue dye is less effective than red or black, the same procedure was followed when blue and red dyes were used.

DISCUSSION

There are too many variables in this type of test to permit valid conclusions. Based on the catches in the 10, 15, and 20 mile traps on the Rio Grande, there appears to be no obvious difference in the ability of the individual flies to disperse. Based on catches on Panal Ranch, it is my opinion that there is a demonstrable difference in the 2 populations of flies in the field. The ecological significance of this difference cannot be evaluated by present trapping methods. Controlled releases of both kinds of flies in infested areas with subsequent collections of egg masses would be required for further evaluation in the field.

Calf Suckle

MILK FORMULA

GUARANTEED ANALYSIS

Crude Protein, Not less than	24.0%
Crude Fat, Not more than	4.0%
Crude Fiber, Not more than	1.0%
Ash, Not more than	9.0%
Added Minerals, Not more than	2.0%
Moisture, Not more than	5.0%

No Animal Tallow

No Antibiotics

INGREDIENTS

Dried Skimmed Milk, Dried Buttermilk, Dried Whey,
Dextrose, Soy Flour, Meat Solubles, Vitamin A
Palmitate, D-Activated Animal Sterol, Vitamin E
Supplement and traces of Iron Sulphate, Copper Sul-
phate, Cobalt Sulphate, Zinc Sulphate, Potassium
Iodide, Magnesium Sulphate, Manganese Oxide.

Net Weight 50 Lbs.

NATIONAL VITAMIN PRODUCTS CO.

3401 Hiawatha Avenue

Minneapolis, Minnesota 55406

Pupae in 8-¹³ days

Meat diet -

① { Use meat and fresh plasma all the way
 { Freq. changes (2 12-24-hr)

② Anti-oxid. & prot AA, vit breakdown

③ Mineral, vit, sol. prot, or AA instead of
 H₂O to diet

Start on meat & plasma, then to meat + 3% egg

④ Ling - liq diet to adults (agar base) 0.5 suckle

Ling - Epir liq

Blood - 6% (5-6)

Suckle - 4% (1.8-4)

Catch - 3% (3-7)

EA - 3% (2-3)

16%

Sucrose 1%

Fat-free milk - too much CHO

Req. RNA

Choline

① The first part of the survey
(the first 10 miles)

② The second part of the survey
(the next 10 miles)

③ The third part of the survey
(the last 10 miles)

④ The fourth part of the survey
(the last 10 miles)

⑤ The fifth part of the survey
(the last 10 miles)

⑥ The sixth part of the survey
(the last 10 miles)

⑦ The seventh part of the survey
(the last 10 miles)

⑧ The eighth part of the survey
(the last 10 miles)

P 679

48 hr l diets B C D, I H, J K are the same
(Table 1) but l. range from 12.4 - 46.9 mg/10 l.

Those on 3 g fish flour (H) show greatest gain as
Mature larvae and approach (K).

Fish flour may be a better finishing diet.

48-72 hr on I+H spent in overcoming
small size of 48 hr l.

P. 681 Inc of whole egg to 79% ^(Table 3) inc wt of 1st stage l
* May mid. value in inc Lipine, Me or other ess AA
in 1st stage l. (or at least the impt of protein qual.)

Combination of suckl & CC

Suppt. diet

Corn-saybean ^{ref} 1 lb ^{DL} Methionine / ton
1-2 lb Lipine / ton

Egg albumin - in starting diet?

Were adults from diff diet evaluated

1. Standard = Horse meat plus diluted plasma
2. Experimental - " " " undiluted plasma
3. " " " " + Proteus Chandler
- 3a. 7 larvae plasma
4. - Same as #1 but egg albumin + amino acids ~~to~~ in water to approx. protein in serum.
5. - Same as #1 but vitamins added to water
6. { 3 Minerals ^{Wessons} ~~Hammon~~ ~~Stoppa~~ - Same as #1 but minerals added to water
7. - #1 with protein, vitamins, & minerals.
8. - Use only prep. in #7 for plasma
9. - Other comb.
10. - Dried blood
11. - #7 with textured soybean protein instead of horse meat { Hot water for adding to Soybean } ~~Make~~ Try 40% Tsb in Hot water as sub for horse meat } Hatch l. on Tsb?

Adequate Cholesterol in all diets

pH Control with buffers

Effect of animal hormones in plasma

Inhibitors - Nephramin Cl, Antibiotic

- Systemic antib. to kill *Proteus* in wound for effect on larvae. Might need to cult. resist strain of *Proteus* to measure effect of antib. on larvae
- Formaldehyde = gen. o.k.

Effect of waste products on larvae

- Anti-oxidants?

4th Central with buffers

Effect of animal hormones in plasma

Adrenaline - epinephrine

- Adrenaline acts to mobilize amino acids

Effect on liver. Mobilizes & will react

Effect of Adrenaline to increase effect of other hormones

- Adrenaline = fight or flight

Effect of growth hormone on hormones

- Growth hormone

Variables to measure

wt. of larvae

Color

Thorax - w/l ratio

Longevity -

SAC test - Std horse meat flies -

Ratio of σ to ϕ

Time to death of 50% of ϕ

killed in a specific time

Effect of pre-ferment diet & B. subtilis on larvae

What host are in spent media

Hodgson - All apt in TLC of sheep & adults may be by type of glycerol. One for the other linkage, other 2 have ester. Two types.

glycerol = all but one on lipid top
glycerol = locked lipid

Effect of initial horse meat during hatch on larvae - Col. Jr.

Color of eyes different in flies from Mass. raising in horse-meat based. Hinton & EP

Miscell.

Efficiency of Conversion of protein = 11% LOW
1000 - 60 mg larvae from 454 gms meat

Amt. NH_4 in wound of living animal
pH inc. from 7.0 to 7.4 as it matures.

Could NH_4 be an attr. for *ScW* Ps?

Robinson
Rutky

{ What is amt protein in spent media
" " eff of waste on larvae
Effect of pre-ferment diet c *B. subtilis* on larvae
What bact. are in spent media

Nadgson - Dblt spt in TLC of sheep l + adults may be 1 type of plasmalogen. One fa has ether linkage, other 2 have ester.
Two types:

glycerol = dbl bond ox on C next to H
glyceryl = lacked dbl bond.

Effect of initial horse meat during hatch on larvae - Bob Ya

Color of eyes different in flies from Mass Raring vs Horse meat reared. Herndon do EP

Initial food of larvae
Agar - eyes

Lt. Bob know about Genetic
Prog. in Fla.

Phormia - Bellan salt mix. - See paper [Hodgson]

Bob Y. - Emph. behavior and means of evaluating it
- Changes in wild popul
- mess comp. of { - Mix Ster. Mass⁹ + Fertile H.M. Anuldetu fert egg
" Fert + Sterile H.M. + later fert egg
this
- Electro physiol resp. to stim.
- Initial food of larvae
E. Hodgson - Agrees on need for method of eval. &
Competitiveness

Henderson - do EP of eyes animal vs Mass Reared?

Bob Y. - Horn worm & polyphag at first, then become
specific for food. Could initial horse meat for
newly hatched Star. SCW & predispose and
slow dev. for adjust to media

A. C. Baumhauer
U.S. DA - ARS
Tox Res Lab
Oxford, NC 27565

Seasonal diff in Se W color

Best success - was with fresh horse meat, & blood
gave 90-95 mg l. - approaches norm 90-120 mg fly

Bact. norm found in wound = Proteus chandleri
resembles *P. vulgaris* in morph & cult but
differs in biol. char.

Lies from animals appear brighter

Young larvae have diff. req. than older ones. = Plasma?

Size inc. with longer feeding

Role of symbiots

Analysis of wound fluid

pH of wound 7.0 @ Hatch, 7.4 @ 4 days
Hormones in blood - JH, DES, TH, etc.

Meat 3 larvae is OK for 3-4 days.

Possible factors

1. In all diet the plasma has been diluted at ~~least~~
 $\frac{1}{3}$. Orig diet 1 kg meat

500 ml Plasma

1 l water

6 ml H₂O

① Protein, ② vitamins, & ③ Minerals are diluted.

Serum has higher percentages of Cy, Le, Ph, Tryp, Tyr

2. pH of wound 7.4; media = ?

3. Proteus chandleri in wound; media = ?

4. Effect of animal hormones on insect dev.?

5. Effect of formaldehyde?

Other bact. inhibitors?

Metabolism - Altman, P. L. + W. S. Pittman

Fed of Amer. Soc. for Exp Biol.

1968

Anti-biotic in animal - elim. Protein
Resist strain of Pr to streptom. effect on larvae

Insect Path
Jane Vaughan

Ck egg fecundity + fertil. -

Ron Goodwin

Add. Proteins to diet -

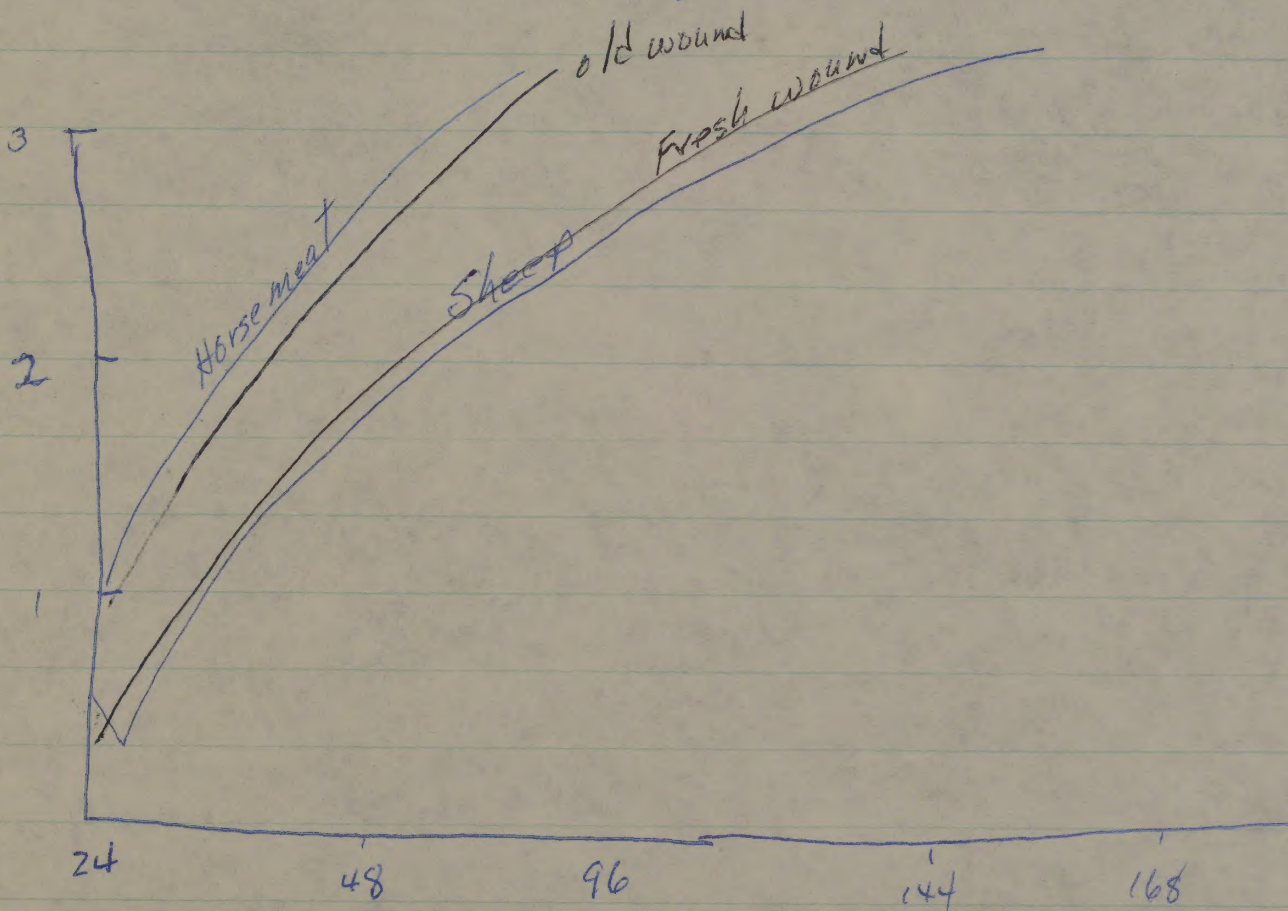
Flight stamina test -

Cor H.M. fly c. Annelids + ratios -

Bill^{R.} - Ant Cholesterol from analysis of milk, dry blood + egg solids

Bill H. - Early larval growth is more rapid on HM than wound.

- Larval growth in prev. ^(old) infest wound is more rapid than in new wound.



From ERD QR

Physiol. fluid = free amino acid

$$6.05\% \times 6.25 = \text{wt of prot}$$

46% sample = protein

$$16g N = 100\% \text{ Prot}$$

$$\frac{6.25}{100}$$

100 g prot

$$.43 \times 16 = 90 \text{ g in Prot.}$$



CHILEAN

NITRATE SALES CORPORATION

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COLUMBIA, S.C. • CALL 256-3556

233-4181

Lapeka, K.

7/15/2006

155367

Seymour Foods

white-ult

80 % protein in ^{spray} egg alb.

913-234-8661

James Carwin

Total protein in flies
conversion to %
values

Dif. in values to be
signif

BW diets for Fern

~~BW diets Exper~~ ^{Ret. spec.} ~~CSF standard~~

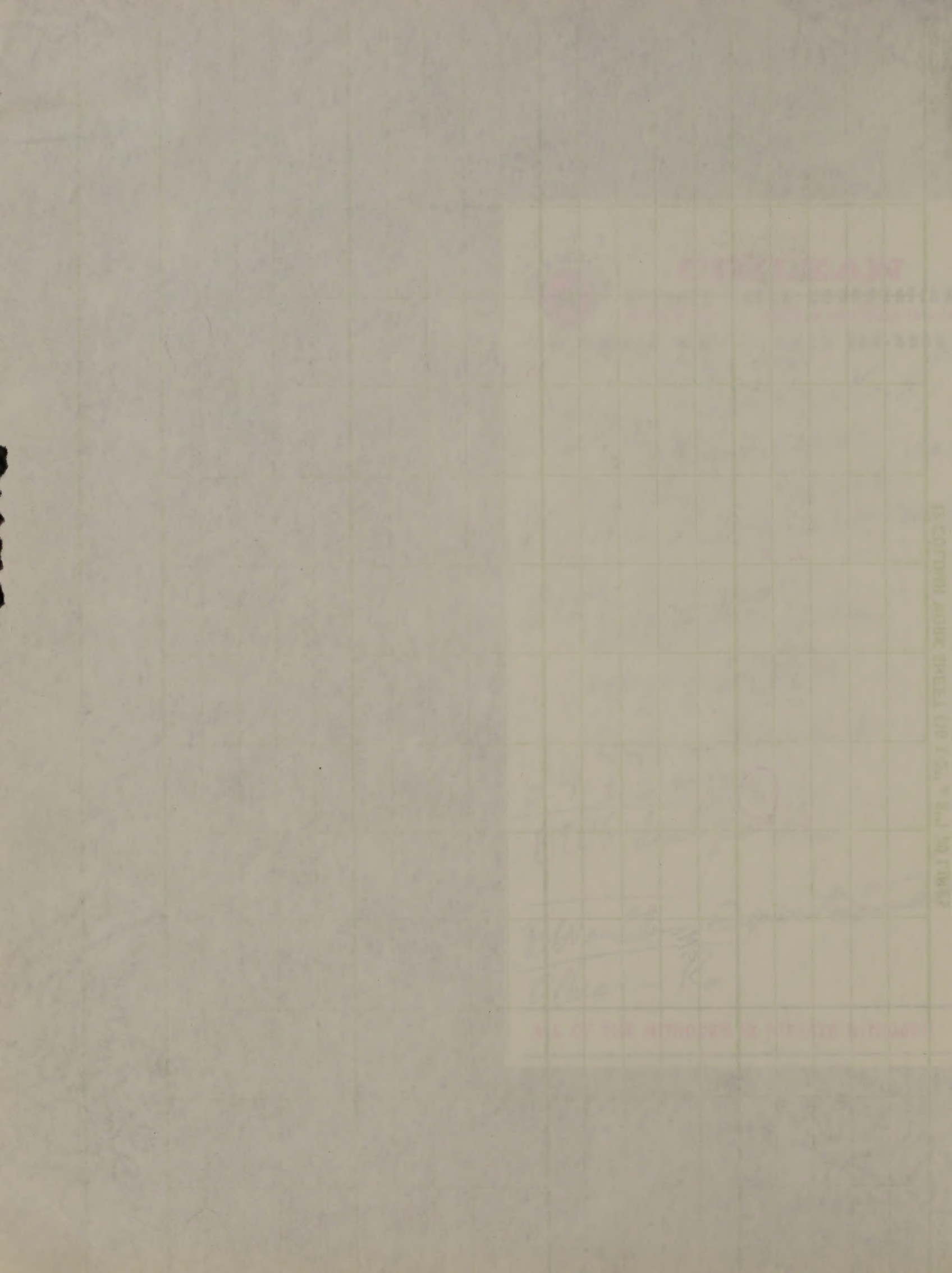
Other - Ro

ALL OF THE NITROGEN IS NITRATE NITROGEN

Sh
adult
♀ ♂ ♀ ♂

Sh
Female
Tx F13
Med Sh

Lg *	.44	.60 ₁₁	.71 ₂₇	.49	.63	.67 ₁₀	.57				
Hi	.19	.23 ₀₄	.27 ₀₈	.19	.21	.20	.18				
Ar *	.35	.33	.47 ₁₂	.34	.28	.27	.25				
As	.74	.79 ₀₆	.74	.73	.63	.86	.86				
Lb *	.32	.31	.30	.31	.29	.33	.31	NS			
L	.32	.31	.31	.32	.27	.35	.32				
Glu	.97	1.013	.97	1.02	1.07	1.08 ₀₅	.03				
Pv	.29	.48 ₀₆	.43 ₁₄	.42	.47	.35	.37				
Hly	.39	.45 ₀₅	.39	.40	.45	.40 ₀₄	.36				
Ala	.50	.61 ₀₇	.51	.54	.66	.36	.37				
Cy -	2v	2v	2v	.04	2v	2v	2v				
V *	.41	.46	.46 ₀₆	.45	.37	.46	.44				
M *	.19	.16	.16 ₀₃	.17	.12	.18	.18				
L *	.35	.33	.34	.34	.28	.32	.31	NS			
Le *	.58	.57	.57	.59	.48	.51	.48				
Iy -	.39 ₀₆	.26	.33	.35 ₀₉	.18	.63	.70 ₀₇				
Ph *	.34 ₀₄	.27	.30	.32 ₀₅	.22	.58	.62 ₀₄				
Iry											



W. A. B. 1917

13 COLUMN GRAPH SHEET (10 x 10, 1/2" x 1/2" S. S. 1/2")

ALL OF THE MINUTES 2 1/2" x 1/2" S. S. 1/2"

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTH CENTRAL REGION
NORTHERN REGIONAL RESEARCH LABORATORY
1815 NORTH UNIVERSITY STREET
PEORIA, ILLINOIS 61604

October 16, 1973

Subject: Amino Acid Analysis of Screwworms

To: R. F. Moore, Jr., Research Entomologist
Pee Dee Experiment Station
P.O. Box 271
Florence, South Carolina 29501

Through: G. E. Inglett, Chief, Cereal Properties Laboratory, NRRL *GED*

Amino acid analysis of screwworm samples has been completed, and the results are attached.

If you have questions about the analysis you may call me at 309/685-4382 (FTS).

James F. Cavins

James F. Cavins, Research Chemist
Physical Properties and Composition Research
Cereal Properties Laboratory

Attachment

$$16 \times 9 \text{ amino acids/g N} = 90 \text{ AA}$$

Amino Acid Analysis of Screwworm Samples

g./g. nitrogen

	<i>adults</i>		<i>adult</i>		<i>adult</i>		<i>Larvae</i>		<i>Sheep</i>	<i>adult</i>		<i>media</i>
	1 <i>sh</i> [♀]	1R	2 <i>media</i>	2R	3 <i>media</i>	3R	4 <i>media</i>	4R	5R	6 <i>sh</i>	6R	7R <i>media</i>
Lysine	.43	.46	.46	.51	.69	.73	.73	.61	.57	.57	.62	.63
Histidine	.18	.19	.18	.20	.26	.27	.19	.21	.18	.23	.22	.21
Ammonia	.14	.14	.12	.13	.20	.18	.14	.11	.12	.20	.13	.12
Arginine	.34	.35	.32	.36	.48	.45	.27	.26	.25	.38	.38	.28
Aspartic Acid	.74	.74	.75	.71	.73	.76	.81	.91	.86	.81	.92	.63
Threonine	.31	.32	.30	.31	.29	.31	.32	.33	.31	.34	.32	.29
Serine	.31	.32	.31	.32	.30	.32	.32	.37	.32	.32	.34	.27
Glutamic Acid	.97	.97	1.01	1.02	.95	.99	.98	1.14	1.03	1.07	1.16	1.07
Proline	.33	.25	.40	.43	.42	.43	.28	.41	.37	.35	.49	.47
Glycine	.39	.39	.41	.38	.38	.40	.38	.41	.36	.49	.45	.45
Alanine	.50	.49	.54	.53	.50	.51	.35	.37	.37	.51	.56	.66
1/2 Cystine	.04	trace	.03	.04	.03	trace	trace	trace	trace	.02	trace	trace
Valine	.44	.42	.45	.44	.44	.47	.41	.50	.44	.45	.54	.37
Methionine	.19	.18	.16	.17	.17	.14	.19	.17	.18	.20	.19	.12
Isoleucine	.35	.34	.35	.33	.33	.34	.31	.33	.31	.36	.38	.28
Leucine	.57	.58	.59	.58	.56	.57	.48	.53	.48	.60	.66	.48
Tyrosine	.38	.39	.35	.34	.33	.31	.66	.69	.70	.36	.34	.18
Phenylalanine	.34	.34	.32	.31	.31	.29	.58	.57	.62	.34	.32	.22

% N (6.25) *folow*

6.05

~~6.60~~

6.60

6.43

10.03

9.27

7.45

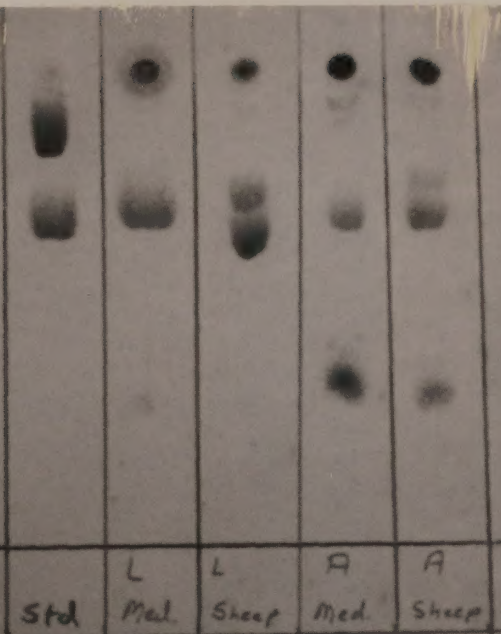
11.7

anal. Ev.
~ 3%

Sample Identification

- 1 and 1R = Sheep Reared (50) Females (7-6-73) 48 hrs. old
 2 and 2R = Medium Reared Males (7-9-73) 48 hrs. old
 3 and 3R = Medium Reared Females (7-9-73) 48 hrs. old
 4 and 4R = Full Grown Medium Larvae (6-28-73)
 5R = Larvae Sheep Reared (6-26-73)
 6 and 6R = Sheep Reared Males (7-6-73) 48 hrs. old
 7R = 48 hr. Larvae Tex. Strain F13 Medium (6-28-73)

6.25 x % N
% protein in sample



Screw worm

8-20-73

D274304N

POLAROID

Hydroponics {Reg 5 days to pupation}

Starting Room {44-46 hrs.}

(24 hr) 1. 200 gm cotton lintus/pan sat. c 5 pt Hydro-lig.
This is smaller pan; in this for 24 hrs.

~~36 hr~~

2. 400 gm cotton lintus/pan sat c 8 pt Hydro-lig.
This is 2x #1. #1 is layered over this dried
egg put around pan to retain larvae. (mix of used & fresh!)

36 hr. MEAT Suppl

Hydro-lig:

6% dried blood, 3% egg, 3% suckle, 3% cottage ch

44-48 hr - Complete H₂O and H₂SO₄

3. Minin supplemental meat feeding at 36 hrs

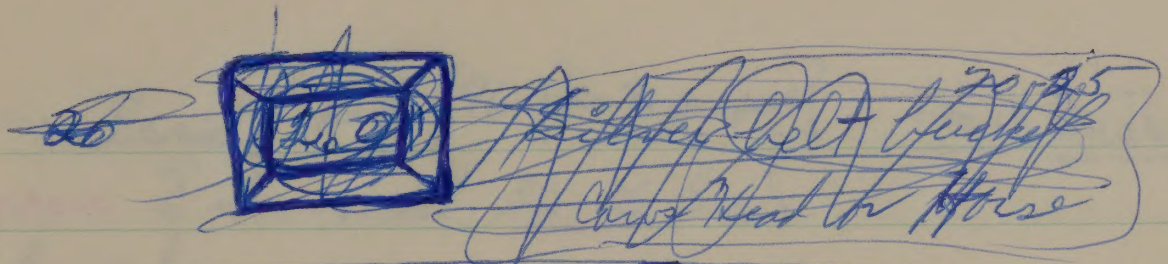
Floors 1 & 2 {76 hrs}

Vats - 4 lbs of cotton lintus sat. c 4.5 gal hydro-juice

Hydro-juice:

6% dried blood, 3% suckle, 3% dried egg, 2% Cottage ch
H₂O & H₂SO₄

144
70
0



~~the~~
Wider the blue color, smaller larger in wild insect
light - mating

Bacterial Contamin of diet is steril. - Inhib. other than HCHO
as ammonia termy
Larger flies c HM suppl. c Cholesterol vit.
Minerals
Protein - Lysine
Me

Fish flour - def in Me, Ly, Le.

L. retarded when switch for meat to HP.

Conc. of Cholest in HM meat diet vs HM

egg - .58% whole	2.32% dried egg
	.07% Ch in "
	.16% inc size h
	.44 Ch in lung
	Pork lung diet. 32%

Send copy travel voucher to
Shelma Jester

For meat or nutria [Reg. 96 hrs to pupae] Frank Dudley

Starting room [on meat 32-36 hrs]

A & f fresh plasma:

Use: 90# meat, 3.5 gal fresh plasma, water HCHO

B Horse meat or nutria:

Use 90# meat, 3% ~~suckle~~ whole dry egg, 0.5 suckle ^{H₂O} HCHO

Dried eggs are inedible, not for Human consumption
fm Marshal Products Co. Egg Products Div, Marshall, Minn 56258

Rearing Floor-

Floor 1, 32 hrs, Floor 2, 32 hours

2700# meat (30x90# chgs), 50 gl. Hydro juice

Hydro-juice: 5% dried blood of 2700#

1% suckle

1.5% of 1% HCHO

Water to proper consistency

General -

If larvae "packed" on hydroponic (Hp) diet then it is too hot or cold or too wet or dry

Consistency of diet is moist - bath in Hp + meat

Hp medium not changed ^{until after} 16 hrs on floor 1 then every — hr.

Used liquid is vac. off (until cotton turns grey) then fresh liquid is added.

Cotton is blanketed over larvae

Has tried torula yeast as sub. for cottage cheese

Larvae are larger in 1st 44 hr than c cottage cheese or meat. but are slightly smaller at end of dev.

$T = 6.04 \text{ g vs } 6.48 \text{ g / 100 l.}$

Adult Lives in complete darkness -

Eggs collected 1x at $5\frac{1}{2}$ days = Selection factor

adults fed honey, water, + mixture ~~spend~~ meat + juices

Onipoint on foam rubber c spent diet as attractant along edges of wooden frame.

Dr. Melvin Crystal - rears & selects new strains

Has developed two Texas strain

New Texas strain - older (NTx)

" " - more recent, fm 18 collections

Texas strains haven't adapted to artificial Diet
ovaries fewer, with fewer eggs esp in MASS
eggs.

Get more and more quickly with Forced Egg
in closed container with meat

Dr. Crystal NTx adults are larger than same insects fm Rearing Pl
Pres. reg. strain is smaller than NT

Dr. Crystal NTx adults have eyes that are more red than those
NTx adults from the rearing plant. Reg. insects
from rearing plant & NTx ^{fm plant} have same color eggs

Some insects have blue almost purple color but
majority are more green.

Baumhoner - JAMA - 12 Kv @ 6-7 day → Min loss in
sexual vigor but 5 days red. sex vigor.

aphis

Plant on liquid media for most of 1971 (Results?)

□ spray dried blood, dry cottage ch, powd. milk, + egg

Heat meat in small dishes for hatching to high T? - Use this in hydroponics?

Vac of spent lig - wasteful? Removes un-used nutr. and leaves some waste prod. in cotton

* Why not use Meat for 1st stage (32 hr)
Req least amt meat @ prob. most critical stage
If nutr. are missing might get sufficient carry-over for 1st stage. Cut off this off of inc. rearing time

Mix lig diet c cme to give it

bulk so vac isn't nec,

(If need support

Does spent media have diff pH. Might find a gum that would gel when pH chgs.

Egg albumin instead of whale egg

Costs of:
Calf suckle

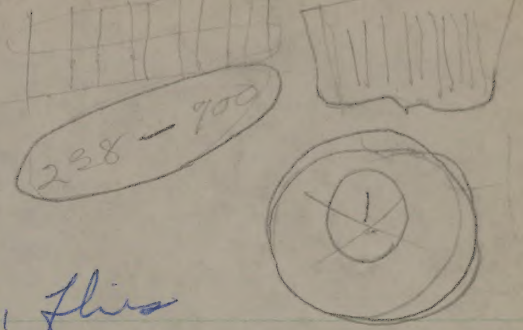
Dried egg

Linters cotton

Dried blood

Cottage cheese

~~Lucke - 17c 14% fat.
 cotc - 20c 40% "~~



Link here in 66-67

Contract AP analysis of HM diet, Hydroponi, flies
 64, 69, 70, 71 best yrs. 63, 68, 72* worst

Larval wts. in mg.

62-71 wts

Lung 62.7, 64.4, 64.4, 65.2, 63.9, 63.3, 64.7

Nutria 62.7, 62.8, 63.9, 62.5

Hydro 52.9, 52.7, 61.7, 66.2,

Norse 64.2, 64.2, 63.7, 64.

Larvae do o.k. on ^{living} g.p.

Hatch - 12-16 hrs. @ 80°, don't hatch @ higher T. ^{well}

67-fall Hurro, 68 high soil moist contrib. SWS, Sun.

62-50,000

63- 1,168, 64-400, 65-1,062, 66-1898, 67-872

68- 9,877, 69-279, 70-153, 71-473, 72-95,000+

71-72 winter weather favorable for SWS sun.

72-Media = Hydro, Lung*, Nutria

Add to HM for Chx + color ??, Vit, Mineral, AA, Bact cont.
 what has been done?

Compos. of feed ^{food} - Minerals, protein, etc.

H. L. House has ^{insect} salt mix.

3rd instar br. ^{big} diet early ~~if~~ for var. of reasons

The Hydrogen-Ion Concentration of Myiotic Wounds in Sheep and Goats

70 @ Hatch
74 @ 4 days

E. W. LAAKE and C. L. SMITH,* U. S. Department of Agriculture,
Bureau of Entomology and Plant Quarantine

The hydrogen-ion concentration of myiotic wounds in guinea pigs and its relation to the oviposition stimulus of gravid females of *Cochliomyia americana* C. & P., the screwworm fly, were presented by the senior author in a paper read at the Thirty-third Annual Meeting of the American Society of Tropical Medicine which was held at New Orleans, La., November 30 to December 3, 1937.

That paper briefly listed the following results: (1) The mean pH of wounds in guinea pigs having lethal and sublethal infestations of screwworms showed a significant increase in alkalinity on the third day of myiasis. (2) For the remainder of the myiotic period the alkalinity diminished only slightly in the case of those having lethal infestations but decreased more markedly with those having sublethal infestations. (3) During the postmyiotic period the mean pH changed to the acid side in the case of all animals that survived, whereas in all that succumbed it remained on the alkaline side. (4) A positive relationship between the maximum attractiveness of myiotic wounds to gravid females of *C. americana* and the period of greatest alkalinity of wound exudates was distinctly indicated.

EXPERIMENTAL PROCEDURE.—The present paper is a report on the determination of the hydrogen-ion concentration of exudates of 32 wounds in goats and of 20 wounds in sheep infested with the larvae of *C. americana*.

The experimental animals were fairly uniform as to age and size. Incisions, 1½ inches long and extending through the connective tissue underlying the skin, were made with a knife or razor blade. Two wounds, one on each side, were located on the rump about 3 inches behind the hip. About 100 first-instar larvae were introduced in each fresh wound.

For the determination of the pH of the wound fluids was made beginning with the fresh (uninfested) wound and continuing through the postmyiotic periods. Wounds

that had closed or scabbed over during the postmyiotic period were punctured or squeezed sufficiently to discharge a sample of the enclosed fluid or pus. Tests were discontinued when wounds had healed to the extent that fluids other than blood were no longer present.

RESULTS OF TESTS.—The results of these tests are presented in fig. 1. A comparison of the trend of the mean pH courses in myiotic rump wounds of sheep and goats shows that these courses are similar except that the changes occur sooner in goats than in sheep. The period of infestation, however, was practically the same in both species of hosts. The alkalinity is comparatively high from the third to the sixth day. This period, and also the peak reached in wounds in both sheep and goats, is of considerably greater magnitude than that reported for myiotic wounds in guinea pigs. This is of distinct importance, since it has been demonstrated with guinea pigs that a high alkalinity in myiotic wounds is highly attractive to gravid females of *C. americana*.

The course of the mean pH of postmyiotic wounds in goats is toward greater acidity as long as pus is present, whereas in sheep it is alkaline or near-alkaline for the entire period. Therefore, since it has been shown that alkaline wounds of guinea pigs are highly attractive to gravid females of *C. americana*, it is reasonable to assume that postmyiotic wounds in sheep are more attractive to the flies than similar wounds in goats.

It is well known that postmyiotic wounds remain attractive for a longer period in some animals than in others. In field work it has been observed that wounds producing a watery discharge do not promptly heal and are exceedingly attractive to gravid blowflies for a long time. Other wounds which are filled with a viscous discharge scab over quickly and are much less attractive to blowflies. In the experiments with guinea pigs it was found that wounds discharging watery fluid are definitely alkaline, whereas wounds of the other type, which scab quickly, are generally acid in reaction.



This information should prove to be of considerable economic importance as a guide in the selection of chemicals to be tested as larvicides, ovicides and repellents. In addition, it suggests a treatment for long-draining or slowly healing postmyiotic wounds—; namely, to acidify the wound by either local or internal medica-

tion. This is confirmed by the results of studies of other septic wounds by Apostoleanu and Vladutiu (1936) and many other physicians who have studied wound healing. Their findings show that acid-producing diets and local acidification give markedly favorable results in the healing of various wounds.—4-11-38.

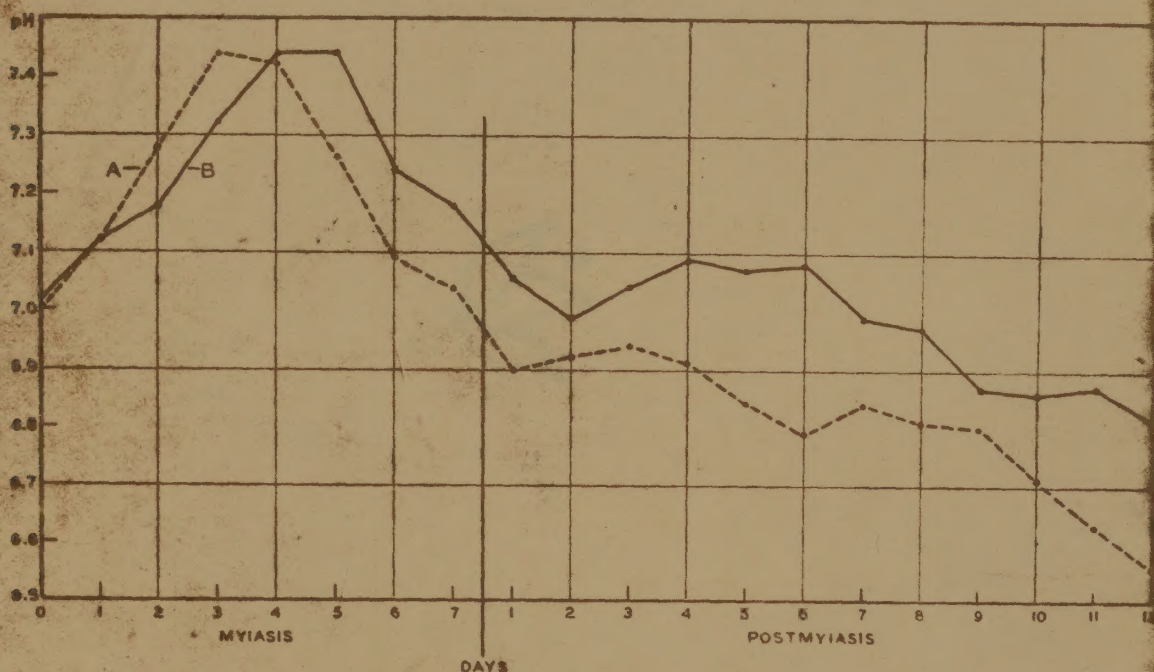


FIG. 1.—Graph showing the hydrogen-ion concentration of myiotic and postmyiotic wounds in (A) goats and (B) sheep.

LITERATURE CITED

- Apostoleanu, E., and O. Vladutiu. 1936. Recherches expérimentales sur les variations du pH dans l'évolution des plaies septiques et en rapport du traitement appliqué. *Lyon Chirurgica* 33: 25-43. Illus.

